

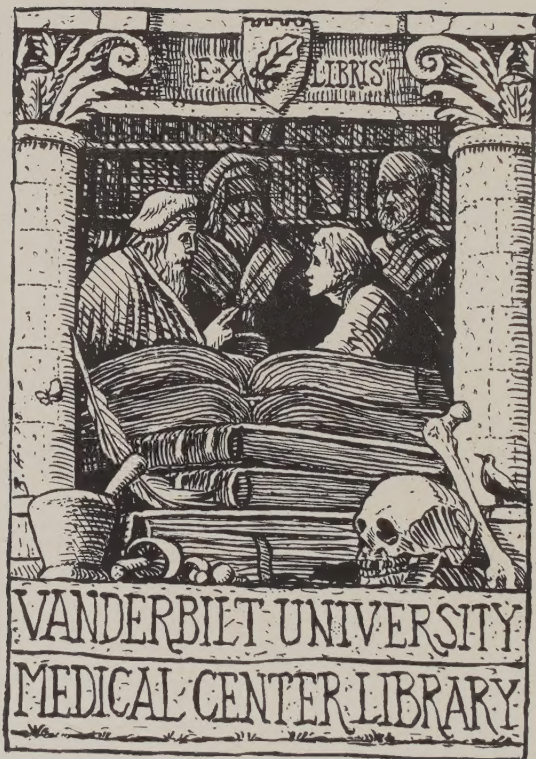
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Internal medicine

Part 1

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EDITORS

L. Condorelli - U. Teodori
A. Beretta Anguissola - M. Sangiorgi

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INAUGURAL
SPEECHES

Inaugural speech

G.A. MARTINI

This society has now its thirtieth birthday. It was founded after the second world war by some worldwide accepted leaders of our profession, mainly European, who tried to save out of the debris of the war some bricks and broken pieces of faith and confidence in order to build a new house, to form a group of friends, a kind of fraternity of physicians whose goal was to preserve and remould the image of internal medicine. Since then, many of the founding members have left us, but fortunately some are still amongst us who were witnesses and active participants of the first meeting. There were some ups and downs between then and now and this society will need all our efforts to keep growing.

During the time of my office I was pleased to watch the growing interest in Internal Medicine in many areas of the world. Invitations to several meetings gave me the impression that even in those parts of the world where internal medicine as such did not exist, a lively interest in Internal Medicine as a subspeciality has developed. The term "internist" was originally derived from the German expression "innere Medizin". It was used in order to distinguish between the surgeons and the dermatologists on the one hand and the doctors on the other. Although this separation and this name is not very satisfactory it will last and it will not be changed to something more exactly representative of what internal medicine encompasses and what internists do. Incidentally this separation is as old as medicine, in fact it was traced back to Homer who wrote in his epic poem *Aithiopis* which is a continuation of the *Iliad*, in the 8th century before Christ about two twin brothers, sons of Asklepios. The one was called Machaon, he was a surgeon, the other Podaleirios, the internist. To each of them Poseidon gave his own fame.

To Machaon, the surgeon, he gave the skilled hands to draw the arrows out of the wounds, to cut and to cure the wounds. To Podaleirios, the internist, he gave the exact feeling, in order to recognize the invisible and to divide the indivisible; to him who was able to read from the fiery eyes of the furious Ajax the deep melancholy.

In many countries, a feeling of crisis in the health care system has developed. The reasons for this are manifold. Although the progress in medicine in all fields is not denied, the care and service for the individual patient or for the entire population has diminished at the same time. The advanced technology has

created a phantastic expectation which medicine has not fulfilled and cannot fulfill.

Medicine, and internal medicine as part of it, are as good as the society in which it moves or in which it is performed and practised. All the problems and difficulties of the society as such, are reflected and even enlarged in Medicine. All the apostles who blame medicine as such for the evils that beset health care will not realize or recognize the profound interdependence of every aspect of modern life.

Who has thought, for instance, of the severe consequences on the daily hospital work, but more so on the whole attitude towards patients' care when the reduction of working hours was introduced?

Hans Popper, who is with us, once concluded that civilisation will only recover from its crisis if freedom and discipline remain connected. We ought to keep open for new developments and combine with it the demand for quality. The most important pre-condition is enthusiasm for the true cause. It is this enthusiasm which we should transplant to the younger generation.

And something more is necessary:

One of the great physicians of our time, William B. Bean of the United States who gave up his Chair of Internal Medicine and has now a Chair of Humanities in Medicine, once wrote to me:

"I have no proof that the return to humanism will regenerate Medicine. But I say unequivocally that the best and finest physicians I have known have been humanists. Adding this dimension will help make physicians, young or old, better people".

Having said this and having stressed the necessity of a broad background which internists should have, it seems necessary, as was recently rightly said, that the role of the internist should be defined and limited, making a clear distinction between what is essential and what is optimal. This role should ideally fit with the needs of the community in which the internist is working. I think it should be one of the urgent goals of this international society to help to define this role and to help those with good advice who want it and who need it. It is here that we should stop and should feel responsible and take up our obligation as *the* medical society which should feel most concerned. We should not try to escape but to take up the challenge:

1. The current reappraisal of medicine should be viewed against this backdrop.
2. The role and training of a general *Internist* should be re-examined, redefined, and its position clarified in comparison with the "subspeciality internist".

3. Postgraduate study has always been a characteristic feature of our profession, said Sir William Osler. Excellence in general internal medicine requires time, experience and mellowing: it requires evaluation of one's performance by others, but more so by oneself!

The last words shall be directed to our Italian friends.

Gli ultimi minuti del mio breve discorso vorrei dedicare ai nostri ospiti italiani e colleghi — pronunciando a tutti parole di ringraziamento cordiale della Società internazionale di medicina interna, della sua presidenza e del suo comitato esecutivo. Si sono incaricati — in questo periodo poco tranquillo — di essere ospiti del nostro congresso. Siamo stati molto lieti che la società italiana di medicina interna ci ha invitati e che la città di Roma ci ha offerto la sua ospitalità.

Sotto la presidenza del nestore della medicina interna italiana, il signor Professore Luigi Condorelli, da noi tanto stimato e onorato, hanno preparato con infinito impegno questo congresso in cui si tratterà un programma assai variato di temi attuali ed importantissimi. Non solamente la medicina interna, ma la medicina in genere deve — sin dall'inizio — alla medicina italiana degli impulsi decisivi. Chi legge l'elenco dei nominativi degli studenti e professori a Padova è profondamente commosso dell'importanza centrale e dell'influenza grandissima per la medicina europea e — partendo da lei — per la medicina mondiale.

Un illustre collega italiano a cui ci siamo rivolti in maniera particolare mi comunicò alcuni mesi fà: La medicina interna? Non c'è una scienza speciale, c'è una filosofia. ✍

Questo non era — per quanto riguarda il congresso — non solamente un consenso affermativo; penso però che faremmo bene a prendere in considerazione molto seria quelle parole e di girare il sarcasmo in maniera positiva, prendendolo per motto del nostro congresso.

Al termine di questo congresso — ne sono convinto — avremo fatto un passo in avanti nella nostra fatica di comprendere di più la filosofia dietro e dall'interno della medicina interna e di renderla ancora più utile per il futuro.

In questo senso tanti auguri per questo congresso.

G. A. MARTINI

President
of the International Society
of Internal Medicine

Inaugural speech

L. CONDORELLI

The participation at the Inaugural Ceremony of this XIVth International Congress of Medicine by top representatives of the Government, Magistrate, Church, University, well-established cultural Institutes, Health and Welfare Service, the Civil and Military Services goes to show how fervent and universal the interest of the Italian people is towards the problems of an advanced culture, and in a particular way towards the progress of medical science.

To all civil, military and religious authorities, whose presence honor this ceremony, go the most respectful greetings and vivid thanks on behalf of the Directive Council for the Society and myself.

In this same historical Capitoline Hall, my dear colleagues assembled together here from every part of the world, there lingers a tradition of a culture which is so precious to all civilized people, and I'm sure you can feel the warm vibrating friendliness which in welcoming you, Rome expresses its universal spirit, whose ancient language still unites intellectuals from every nation; Rome, generous mother that welcomes and nourishes spiritually, without discrimination of race, nationality and creed, all needed in strengthening the soul and intellect.

To the illustrious President of our Society, Prof. Martini; former President, Prof. Vanotti; Mrs. Nanna Swartz, who is among the deans of our Society; and to all the members of the Directive Council, a cordial greeting of welcome on behalf of the Organizing Committee of the Congress and myself.

To all fellow colleagues and their families, I extend with a warm welcome the most profound best wishes for a rewarding task and a peaceful, satisfying stay in Rome.

Appropriately occurring at the present time is the 30th anniversary of the foundation of our Society, which took place in Basilea. The animator of the initiative was Alfred Gigon, who organized an International Meeting of clinical physicians, in which a well-developed scientific program was unfolded and the constitution of the Society was agreed upon, which had the happy beginnings of a party for friendship and international collaboration. There were forty-seven colleagues present from fifteen European nations, China, Israel and the United States of America. Unfortunately, a great majority of them isn't with us today; it is dear to me remembering the high quality of understanding, humanitarianism, enthusiasm and kindness with which together they collaborated to form the foundation of the Society, which held its first Congress in 1950 in Paris.

The Congresses of our Society are associated spiritually to those International Congresses of Medicine, first held in Paris in 1867 in occasion of the International Exposition under the presidency of Bouillaud, and the XVIIth, and last, in London in 1912. The XIth took place in Rome in 1894 under the presidency of Guido Baccelli. The First World War interrupted the series of those memorable international meetings of Medicine.

No association was ever formed: the choices of place and president for each Congress were decided at the end of the previous one. Only, in 1909, in occasion of the XVIth Congress in Budapest, a decision was made to create a Central Commission at The Hague, with the task of communicating between the various nations towards the organization of the Congresses. F.K. Wenckebach was President. Even this Central Commission was interrupted in 1914 with the start of the war.

In the group of those Congresses formed by the Section of Internal Medicine, sections of all branches of Medical Science were present to affirm that the general Medical Clinic is the disciplinary mother which for 24 centuries has provided for the united conscience of the remainder, sprouting as branches from her stump.

When our Society was formed in 1948 with the sole intention of restoring the custom of periodical international medical encounters, the impossibility of riuniting the devotees of all specialized sectors born out of chapters of Internal Medicine was also understood.

These sub-specialties, forever essential parts of the disciplinary mother have become subject to special research of devotees particularly skilled to the technological aspects of diagnostical and therapeutical applications: in which the concept of "specialty" must be understood. And because of this, it was wished that our meetings, restoring the function and tradition of the General Congresses of Medicine, should bestow upon their scientific programs amplexity capable of embracing all fields of internal medicine, which every special aspect up to the most minute detail would be considered and treated as "*sub specie totius*", to the point of always keeping present the Hippocratic concept of the unseverable, psychic and somatic unity of Man under the conditions of health and sickness.

* * *

In an atmosphere of high spirituality and friendly agreement, we'll spend four days of intense work, dear colleagues, which will re-seal old friendships and will cause new avenues of respect and human sympathy to develop among the studious from far-away lands made brothers by common ideals of truth and kindness.

In setting about towards the development of a well-fed scientific program—in which it tried not to leave out any part of the great knowledge of internal medicine that although constituting the body of doctrine for many specialized disciplines—it's wise, for end results, not to lose sight of the singlemost concept that must guide us in intensely treating particular problems, stopping to think of some of the ethical aspects of medicine regarding the procedure and education of physicians.

The scientific research in medicine transcends the simple curiosity of knowing. Medicine, in fact, has as an objector Man's understanding of his undivided spiritual and physical self to the point of defending and restoring health: to attain this highest point, which makes Medicine superior in respect to all other biological disciplines with which it has many similar aspects, it harmonizes and conciliates knowledge extracted from humanistic disciplines which study all that is subjective in Man and from natural science, which investigates the objective phenomena in the physical and biological world.

It's precisely in the solution of the apparent contrast between “*studia humanitatis*” and physical and natural sciences that Medicine finds the reasons for its authoritative position among the sciences in general and biology in particular from which it differentiates itself simply because of its ethical-humanitarian finality.

In fact, still prevailing in the practice of Medicine is the act of willfully adjusting to transform a harmful effective reality, i.e. sickness, into a new favorable reality, i.e. recovery, according to the finalities of who is operating. However, there can't be nor mustn't be dualism, distinctions and, even more so, contrast between theory and practice in Medicine. Theory and practice aren't contrasting, as less learned laymen would like to claim: mutually integrated so that they are solidly united to make it difficult to discover which is the principle in directing the action of a physician.

Moreover, the Art of Medicine is guided by, looked upon and simplified by the feeling of human sympathy and Faith, in the light of which the study and cure of the sick are acts of love. This Art is fed by the noble social desire to better the health of the people, elevating their physical and moral powers, prolonging the duration of a lifetime and profitable work towards progress in civilization.

These are the reasons for which whoever undertakes the study of Medicine, even if he'd later want to exclusively dedicate himself to scientific research, he must be furnished with particular moral powers from which is fed the vocation of the medical profession. Powers that are diligently cultivated by Professors in undergraduate and postgraduate courses, and then continuously sharpened with professional practice and again with uninterrupted study throughout life.

That which is the source of the great spiritual gift of more understanding for donating more towards the sick.

Such moral powers are: wisdom, liberality, friendliness and the spirit of sacrifice.

Wisdom at the moment of a determined act from which therapeutic idea—action derives from, identifies itself with prudence and decision that are essential requirements for justly foreseeing, providing and operating timely.

Liberality bestows upon the physician the spiritual pleasure of donating to the suffering the wealth gained though extensive studies along with the Professors' teaching and by personal experience: he's always incited to understanding more, to the point where he's able to practice the "ars medendi" with the most efficiency.

Friendliness is the gift necessary for opening minds of the patients where personality is to be studied. Hippocrates warned (*De ornatu decete*, chap. 7) that the lack of friendliness prevents the physician from communicating with the healthy and the sick.

And then, the *spirit of sacrifice* is an absolutely essential gift in the practice of the medical profession. "Among the practical skills"—said Hippocrates—"there are those which become distressing to whoever is practising them and actively good to those who have need of them, and constitute common good for the community. Among these medicine stands out. The physician, in fact, sees horrible things, feels the sadness from the ills of his neighbor while the sick take comfort towards their sufferings with the medical skill; that can also remove death from them".

All the forementioned gifts have a fundamental of goodness, without which there wouldn't be a vocation in Medicine. They are at an almost potential state in the young: it is expected of the Professors to single them out in the student and develop them. This is the fundamental for the teaching of the Medical ethics which can't be imparted through theoretical institutional teaching. The ethics of Medicine being so closely linked to the practical activity, there can't be understanding without studying, assisting and curing the ill under the guide of the Professor. And such teaching is above all the assignment of Clinical physicians that contribute in a prevailing way to the formation of the medical personality in students.

The ethics of Medicine are taught, therefore, in the wards of clinic departments making the students participants in the study of patients, which doesn't only concern with physical sickness but also moral suffering. The students become associated with the Professor in choosing the diagnostic findings and therapeutical procedures with attentive care and applying them with all the precautions of the skill, avoiding any such act that might even slightly offend

the patient's character. The importance of moral resources in combatting physical illness is taught and revealed to the students, and how much and in what way the doctor must operate, in order to keep it high in the patient being attended to. Above all it's taught by example how the anxieties of the family are respected and praised as they anxiously look after their dear one's health, and with which feelings of human interest has to be shown to comfort them at painful moments in which sad news are passed on concerning the health of relatives. One must, by example more than by words, exalt the spirit of sacrifice and Christian charity that must assist the doctor in the sight of human misery, even the most hostile. By example still, the strengthening of mind must be inspired in the students that will support them in the sight of torturing scenes of pain.

So consisting the teaching of medical ethics, it is easy to understand the difficulty in passing it on effectively in the overcrowded universities in which human contact between Professor and students and between students and patients is almost lost.

For this reason of the present decline in the teaching of medical ethics in the universities, another of minor importance has been added: the yielding of acceptance to Faculties of Medicine in many countries, Italy included, of young men with secondary cultural preparation exclusively technical (and not always valid), so lacking of human culture to make the formation of the personality difficult, not only that of a great researcher but of a good humanitarian doctor.

But even the sharpening of the doctor's personality with professional practice after university studies is hindered by the advent of "Mass medicine" which slows down the human relationship between doctor and patient, humiliates the personality of the unhealthy Man and bestows upon the physician the appearance of an employee making the most human of the professions bureaucratic.

The ethical teaching of physicians concerns itself with clinical experimentation as well as assistance to the sick.

The clinic, as any biological science, has naturalistic avenues: it is an aspect of learning through a methodism directed towards the study of natural objects and facts and to the research of their genetic linkings, not in abstract order, but rather concrete as history.

The revolution derived from the penetration of Galilean thinking in the biological science methodism and brought on by Morgagni in Medicine with the memorable prologue. "Nova idea institutionum medicarum" of 1712 isn't that which substituted the historical-descriptive method with the experimental one, but that which introduced the *measure* in the study of biological phenomena carried out with the strictness of physics, chemistry and mathematics.

The experiment of clinical physico-pathology is carried out with the same

scientific strictness as those concerning experimental physiology, but with techniques of which will constitute in no way an even minute danger for the patient.

Of the pathological phenomena on which one investigates, and not only in the "snapshot" of that moment of the observation, but in the *preceding* and *processing*, one tries to individualize all the conditions that compete with its genesis.

But such conditions aren't put out in the open by us, but by Nature. And when we must verify to the proportion of the precise experiment, if all the conditions that we judge competing with the origin of the event are necessary or sufficient, and if eliminating or altering doesn't cause the event to be verified or modified, then we must patiently await that in an upcoming clinical case Nature will offer us that experimental situation suitable for solving the problem. The situation or condition that for us Doctors is not permissible to create artificially in Man to the same proportion with which the physiologist operates with animals.

And then, besides the consideration that, by experimenting, it's never possible to create conditions analogous to those that we need to observe in the spontaneous illness. It's therefore inherent to the same nature of the clinical experiment the reason for which it is much more difficult and longer, as compared with that on animals: given that a diligent study and finesse of wisdom is needed to individualize all the "conditions" placed by Nature so that the spontaneous pathological phenomenon is to take place. And it's necessary to collect a great number of observations to be able to recognize at the end, among the most varied combinations that the single cases offer for consideration, which of those conditions really compete with the genesis of the event subject to investigation.

These are the ethical and methodological characteristics that clearly differentiate the clinical experimentation from that of experimental physiology and pathology. And this experimentation is not only legal, but essential for the progress of science: it is as a matter of fact the essence of clinical research.

I wanted to keep completely separated, in these ethical considerations, the physio-pathological experimentation on Man from that of pharmacology. The ethics of this latter experimentation are part of the first dawning of medicine: it is synthesized from the maximum "primum non nocere". This being a commandment which however is often unconsciously lacking today, under the pressure of the continuous and plethoric introduction in the therapeutic outfit of chemical substances of which complex pharmaco-dynamic actions and latent toxics aren't studied sufficiently enough before the concession of use. The incautious use and wide untimely therapeutical application of remedies of which all the effects present and remote aren't still well-individualized, have painfully

created the recent, vast, unexpectedly different, and rapidly evolving chapter of the “Hiatrogenic diseases” that constitute the most mortifying offense for medical ethics.

The very rapid present progress of the means of instrumental investigation applied to biology and medicine confers, with modern technical texts of the specializations born out of various chapters of Internal Medicine, a duration of some decade. But the general methodology of “*Ars medendi*”, which is the essence and the synthesis of all the sciences turned to the guardianship of Man’s health, and ethics to which we must turn to in inspiring our professional action, seal eternal truths, superior and independent from technical progress.

I’d like to close my speech nevertheless, dedicated above-all to the younger of our brethren, future Professors of Clinical Medicine, with the always present words that Andrea Cesalpino, dismissing the “*Ars Medica*” in the same year of his death (1603), turned to the students of his course on “*Medicina Sovraordinaria*” at the Sapienza. Words, solemn as a pledge, which gather together the sublime ethic principles of science, art and teaching of Medicine: a moral guide for physicians of past and future generations.

“You that have chosen to practice the Art of Medicine, be just donors of this gift of God, and to no one, especially the needy, refuse it for lack of profit. Study yourselves so as not to offer at anyone the occasion to despise this art because of your unskillfulness or habits. And thus, without doubt you’ll succeed if all that science has taught you will be put to use, never to damage anyone but always to heal the sick, and if you’ll do all that’s possible to preserve Man’s health.

Don’t place in jeopardy human health with reckless experiments, or by applying rash doctrines: but gather from the most reliable Authors faithful observations and correct judgements. Consult these so called Authors with diligence, lest you might suffer the blame when you weren’t able to restore the health to your patients.

Don’t even believe me, your Professor, if you see me taking false avenues, since there is still no man, and more or less also I, on whose authority you may blindly cure a human creature without suffering the blame”.

Remind yourselves, furthermore, that you have sworn not only to the solemn oath of Hippocrates that the ancestors lent to the pagan gods, but to our True Lord, God Almighty”.

L. CONDORELLI
President
of the Congress

LECTURES

Molecular pathology in internal medicine

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Molecular diseases are defined as the disorders caused by abnormality in the primary structure or amino acid sequence of a protein in the living body.

The concept of "molecular disease" has been established by a number of studies on structure and function of abnormal hemoglobins and on pathophysiology of the hemoglobinopathies. However, molecular diseases are not confined to hemoglobin (Hb).

In this lecture, molecular diseases are reviewed with particular reference to clinically important problems from the recent progress of abnormal hemoglobin research.

Hemoglobin is a heme protein packed in the red cells, the main function of which is to transport oxygen to the cells of organs and tissues. Each molecule consists of four polypeptide chains, two pairs of α and β chains with one molecule of heme in each chain.

Hemoglobinopathies

The hemoglobinopathies are defined as the diseases caused by inherited structural abnormality of the protein part, globin, of hemoglobin. They are clinically classified as follows.

1. *Hemoglobin S and Sickle Cell Anemia.* Sickle cell anemia was the first model of hemoglobinopathy to be designated as the molecular disease. It is a severe hemolytic disorder caused by the homozygous occurrence of Hb S. Hb S, $\alpha_2\text{A}\beta_2^{\text{Glu-val}}$, having normal α chain, but abnormal β chain, the glutamic acid in the sixth position from the N-terminal being replaced by valine, aggregates easily in deoxy form and decreases in solubility, forming the sickle type red cell.

Due to the presence of Hb S, the well-known various clinical manifestations are shown in these patients.

It should be stressed that the variety of clinical manifestations are derived from Hb S, which has a single amino acid substitution due to a point mutation in structural gene of β subunit. Hb S is considered to be the best example of the pleiotropic expression of the gene action.

2. *Unstable hemoglobin*. So-called Heinz body of red blood cells is often derived from unstable hemoglobin. The clinical features vary from variants to variants, some having almost no symptoms, while in others the degree of hemolysis is much more severe, accompanied by jaundice, splenomegaly and pigmenturia (Koler *et al.*, 1973).

The instability of Hb molecule of this group is caused by amino acid deletion, substitution to a prolyl residue or substitution involving one of the residue in contact with heme group, in the interior of the molecule or on the surface of $\alpha_1\beta_1$ or $\alpha_2\beta_2$ subunit contact.

The patients having unstable hemoglobins present a beautiful model for the relationship between health and disease due to molecular abnormalities.

3. *Erythrocytosis producing hemoglobin*. A group of abnormal Hb is characterized by the increased oxygen affinity followed by the secondary erythrocytosis.

These variants have amino acid substitution at the sites crucial to hemoglobin function, i.e. affinity of Hb for oxygen. In these abnormal hemoglobins, amino acid substitution occurred at the position of $\alpha_1\beta_2$ intersubunits, around C-terminal area of β subunit and the binding site of 2,3-disphosphoglycerate.

The very high affinity of Hb for oxygen causes a severe tissue hypoxia, enhancement of erythropoietin production and induction of polycythemia (Adamson *et al.*, 1972). Erythrocytosis due to abnormal Hb allows us physiologically important insight into the mechanism of oxygen binding, its delivery and hematopoiesis.

4. *Hemoglobin M*. As shown in Table 1, Hb M disease is characterized by cyanosis and, in some variants, mild hemolytic anemia. Of five types of Hb M in adults, four were already found in Japan. In each of them, one of the two

TABLE 1
Hemoglobin M variants

Hemoglobin	Substitution		Location	Cyanosis	Hemolytic anemia
M Iwate	$\alpha 87(\text{F8})$	His \rightarrow Tyr	Heme	Definite	Absent
M Boston (Osaka)	$\alpha 58(\text{E7})$	His \rightarrow Tyr	Heme	Definite	Absent
M Hyde Park (Akita)	$\beta 92(\text{F8})$	His \rightarrow Tyr	Heme	Less definite	Present
M Saskatoon (Kurume)	$\beta 63(\text{E7})$	His \rightarrow Tyr	Heme	Less definite	Present
M Milwaukee	$\beta 67(\text{E11})$	Val \rightarrow Glu	Heme	Definite	Absent
FM Osaka	$\gamma 63(\text{E7})$	His \rightarrow Tyr	Heme	Definite at birth faded away at 6 months	

histidines in contact with the heme group in either α - or β -chain is replaced by tyrosine. Thus these Hb M are structurally homologous in relation to the heme iron to which histidine is coordinated although Hb M Milwaukee is different from them.

We had the opportunities to study the physicochemical properties of all five variants (Hayashi *et al.*, 1969). The study of reactivity to ligands revealed that the heme iron in abnormal subunit of Hb M was in ferric state because iron formed a stable internal complex with the substituted amino acid and that normal β subunit of Hb M Boston and M Iwate remained in deoxy form even under ordinary atmospheric oxygen, resulting in more marked cyanosis than other Hb M.

It was particularly interesting that the electron paramagnetic resonance (EPR) spectra of Hb M showed remarkable abnormality (Hayashi *et al.*, 1966). The shapes of signal are characteristic to each variant of Hb M. The use of EPR is recommended as a simple and accurate diagnostic procedure for the identification of each Hb M disease.

A new variant of Hb M has recently been discovered in blood from a premature baby who had severe jaundice and cyanosis (Hayashi *et al.*, 1978). EPR spectra of her blood was almost identical with that of Hb M Saskatoon. However, column chromatography of her hemolysate on Amberlite CG 50 demonstrated that she had a new M variant of Hb F. It was tentatively named Hb FM Osaka. The chemical structure of Hb FM Osaka is now under investigation. Six months after birth, most of Hb FM Osaka was replaced by normal Hb A and she is growing up in a good condition. The switching phenomenon from γ subunit to β subunit is considered to have normalized abnormal hemoglobinopathy.

5. *Asymptomatic abnormal hemoglobin.* There are many abnormal hemoglobins which are not associated with any disease or symptoms. Most of these abnormal hemoglobins have the abnormality on the surface of molecule. Since it should be very difficult to detect them, it is predicted that there are tremendously large numbers of abnormal hemoglobins, although more than 250 variants have been reported at present.

Molecular pathology of hemoglobin

Molecular pathology of human hemoglobins deduced from researches of hemoglobinopathies are summarized in Table 2.

As a rule, the amino acid substitution on the surface of Hb molecule brings about neither functional modification nor clinical manifestation. One of a few exceptions is sickle cell anemia caused by Hb S.

TABLE 2

Molecular pathology of abnormal hemoglobin

<i>Locus of replacements</i>	<i>Morphological and functional changes</i>	<i>Clinical manifestations</i>	<i>Example</i>
Surface	None except for Hb S	None except for sickle cell anemia	Hb Nagasaki
Internal	Unstable	Hemolytic anemia	Hb Savannah
Heme contact groups	Unstable	Hemolytic anemia	Hb Köln
Iron contact groups	Formation of methemoglobin	Cyanosis	Hb M Iwate
Contact between subunits			
$\alpha_1\beta_1$ (or $\alpha_2\beta_2$)	Unstable	Hemolytic anemia	Hb Tacoma
$\alpha_1\beta_2$ (or $\alpha_2\beta_1$)	high O ₂ affinity	erythrocytosis	Hb Chesapeake
Around	High O ₂ affinity	Erythrocytosis	Hb Hiroshima
C-terminal			
Others	Formation of aggregates	Sickle cell anemia	Hb S

The abnormalities in intramolecular part of subunits, around heme groups or on $\alpha_1\beta_1$ or $\alpha_2\beta_2$ intersubunit surface leads to the instability of the hemoglobin molecule functionally and hemolytic anemia clinically. M hemoglobinemia has an abnormality at the position very close to heme iron revealing cyanosis. Erythrocytosis is caused by abnormalities on $\alpha_1\beta_2$ or $\alpha_2\beta_1$ intersubunit surface, around carboxy terminal group or at diphosphoglycerate binding sites.

Thus clinical manifestations could be explained clearly on the basis of functional and morphological changes due to the abnormalities of highdimensional structure of Hb.

Substitution, deletion and variation of amino acid in the subunit of Hb, which cause the abnormalities of molecule, are brought out by genetic alteration. It is very interesting and exciting that the molecular biology of gene action worked out in microorganism such as E.coli and bacteriophage can explain the genetic mechanism leading to the production of human abnormal hemoglobins without any controversy. Point mutation, crossing-over of gene, frame-shift mutation and their combination give rise to the abnormalities of primary structure of Hb and clinical manifestations of hemoglobinopathy.

Molecular disease in internal medicine

Molecular diseases are, of course, not confined to abnormal hemoglobinemia. An increasing number of molecular diseases are being reported, including glucose-

6-phosphate dehydrogenase abnormalities, Lesch-Nyhan syndrome, Tay-Sachs disease, Gaucher disease, and so on.

Consequently, general concept of molecular diseases is becoming more and more important in elucidating the pathogenesis of diseases. The knowledge of molecular diseases might be applicable to the metabolic and immunological diseases in general as well as the inborn errors.

It should be realized that the study of genetic background is extremely important for understanding of etiology, pathophysiology and prevention of the diseases. The concept of molecular disease will contribute greatly to internal medicine in future.

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Myocardopathies of unknown etiology (Primary cardiomyopathies)

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According to the recommendations of the Group of study for myocardopathies of the International Society of Cardiology, as primary cardiomyopathy or simply cardiomyopathy must be intended "a disorder of the heart muscle of unknown cause or association" (Oakley, 1971). The term "cardiomyopathy" (CMP) that I shall use in my paper, is commonly employed in the anglo-saxon literature, but it has the same semantic meaning as the term "myocardopathy", preferably used in European, especially in Italian and French literature.

This definition emphasizes the concept that in cardiomyopathies the myocardial lesion is a primary event, or at least apparently such, that is independent of any other previous general or cardiac recognizable affection (Goodwin, 1972; Lenègre, 1972).

Therefore the definition given by McKinney in his recent volume on the pathology of myocardopathies (1974) must be refuted. He defines as primary cardiomyopathies not only those of unknown etiology but also those of known etiology in which the heart only is affected by the morbid process, whereas as secondary cardiomyopathies are considered those heart diseases in which the myocardial disease is connected with a more or less generalized morbid affection. This would bring about a further confusion in this chapter of cardiac pathology, for many aspects still very controversial.

It appears therefore convenient to distinguish largely between myocardopathies of unknown etiology or primary CMP in which the disease affects clinically and usually only the heart and specifically the myocardium, and the group of myocardopathies secondary to known causes, including a vast series of general diseases or of diseases of various organs and apparatus among which the myocardium.

The chapter of primary CMP has been particularly developed during the last twenty years, since the unusual progresses of the cardiological technology—especially with cardiac catheterism, ventriculography, coronarography, echocardiography, cardiac biopsy—as well as the progresses of the clinical and laboratory diagnosis have generally permitted to obtain data of greatest importance for the recognition and for the physiopathological study of many forms of car-

diopathies which before were included with exasperating monotony—and unfortunately if often happens still nowadays—in the large cauldron of ischemic, hypertensive or rheumatic heart diseases.

In the comprehension of this group of affections a remarkable progress was reached by the classification of Goodwin and Oakley (1972) in congestive CMP (characterized by dilation and kinetic myocardial insufficiency), obliterative CMP (restrictive, constrictive), very rare (characterized by obstacle to ventricular filling) and hypertrophic CMP (characterized by hypertrophy, impaired diastolic compliance, possible obstacle to left ventricular ejection).

Substantially nothing has been changed by other more recent hemodynamic classifications (Geschwinde *et al.*, 1974; Rudzyllo *et al.*, 1976); on the basis of angiographic-hemodynamic correlations, a distinction is made mostly between a hypertrophic-hypokinetic form (corresponding to Goodwin's congestive form) a hypertrophic-obstructive form, a hypertrophic non obstructive form and a mixed hypertrophic-hypokinetic form which would constitute an evolucional or a transitory form from hypertrophic CMP to the congestive one.

Nevertheless it should be pointed out that these anatomic-hemodynamic classifications, concerning exclusively or prevailingly the pathology of the contractile function of the heart, i.e. of the heart as a pump, exclude from the primary CMP other forms which, on the contrary, concern exclusively or prevailingly the pathology of the specific conducting system and which are today well known: I refer in particular to the atrioventricular and intraventricular blocks and other arrhythmias and to electrocardiographic anomalies of the ventricular repolarisation, of familial character; they may often present important problems of differential diagnosis as regards hypertrophic CMP, these, too, for the most of familial character and frequently associated with ventricular blocks and different arrhythmias. Moreover, among the forms involving the contractile myocardium, besides the hypertrophic there exist certainly some sclero-atrophic forms, they also of congenital or familial character, such as the papyraceous degeneration of the right ventricle (or Uhl's disease) and the idiopathic right atrial sclerosis.

Thus it is possible to distinguish on one hand the group of congestive CMP, sometimes congestive-constrictive, non familial, and on the other hand a group of familial CMP, probably of dystrophic origin, maybe due to enzymatic disorder of the development and trophism of the myocardial tissue, affecting the contractile myocardium (of sclero-hypertrophic or sclero-atrophic type) or the specific conducting system.

I believe that a more comprehensive physiopathologic and clinical classification of the primary CMP might be the one I proposed in 1974 (Table 1). According to this classification the definition of primary CMP should be modified

TABLE 1

Physiopathologic-clinical classification of primary CMP (Sangiorgi, 1974)

-
1. *Congestive CMP* (sometimes congestive-constrictive): apparently primary, probably secondary to multifactorial unknown noxae, non familial.
 2. *Dystrophic CMP*: true primary cardiac myopathies, for the most congenital or familial.
 - a) of the contractile myocardium:
 - hypertrophic CMP, obstructive, non obstructive (left ventricle, rarely right ventricle);
 - sclero-atrophic CMP (right atrium, right ventricle, left ventricle);
 - b) of the conducting system:
 - familial blocks and other arrhythmias, pre-excitation;
 - familial anomalies of the ventricular repolarization.
-

as follows: "CMP are cardiopathies due to a primary impairment of the contractile and/or specific myocardium of undefined or unknown origin and anyhow not secondary to general diseases or to known pathogenic causes (Sangiorgi, 1974, 1977).

It is not easy to establish the real frequency of these heart diseases, because unfortunately there have not yet been reached unanimous views in this respect and neither have been established uniform criteria of clinical and anatomic-pathologic evaluation.

On the strength of the most accredited anatomic-pathologic and clinical casuistries (like those of Gore & Saphir, 1947; Perrin, 1960; Sackner, 1961; Corsi, 1964) it may generally be assumed that primary CMP constitute 0.5-5% of all of the cardiopathies.

In the course of this lecture I intend to examine shortly the main primary CMP, emphasizing the latest achievements from the hemodynamic, physiopathologic and pathogenetic point of view. This will be a general synthetic review which in my opinion may be more useful for an internistic auditorium than the deepening of specialistic aspects of several single forms which, on the other hand, would be impossible for the time at my disposal.

Congestive cardiomyopathies

From a clinical point of view we can certainly distinguish several forms of congestive CMP, nosographically rather well characterized: 1) a chronic idiopathic form; 2) an acute myocarditic form; 3) the peri-partum CMP; 4) tropical CMP.

The anatomic-pathologic and clinico-hemodynamic characteristics of these different forms are quite uniform and consist in a more or less remarkable enlargement of the heart owing to dilation of its cavities, whereas the parietal reactive

hypertrophy (eccentric hypertrophy) is relatively modest; signs of kinetic myocardial deficiency (atrial and especially ventricular galop rhythms, systolic murmurs due to mitral and tricuspidal incompetence); atrial and ventricular arrhythmias, especially bathmotropic, like atrial fibrillation; pulmonary or systemic embolisms; progressive, acute, subacute or chronic evolution towards terminal cardiac insufficiency with proper signs of right and/or left congestive failure (dyspnea, cyanosis, peripheral oedema, hepatic and pulmonary stasis, tachycardia, hypotension, especially systolic, sometimes diastolic hypertension and reduced differential pressure).

The most important data of the hemodynamic and polygraphic study are those typical of the hypokinetic myocardopathies; they are represented by a more or less remarkable decrease of the systolic stroke volume and of the cardiac output, increase in the right and left telediastolic ventricular pressure and in the atrial pressure, increase in the isometric contraction time ICT, and in the tension time TT, decrease in the ejection time ET, increase in the TT/TE ratio, decrease in the ejection fraction. The curve of ventricular pressure is characterized by slow ascension, a triangular plateau with a rather delayed peak and elevated telediastolic pressure.

The heart is more or less remarkably enlarged with a morphology similar to a "rugby ball". The ventriculography puts well in evidence the dilation of the cavity and the scarce volume difference between systole and diastole because of the remarkable hypokinesia. Also the myocardial scintigraphy with Tallium 201 demonstrates well the dilation of the cavity with reduced thickness of the walls. The echocardiography, too, is very useful for demonstrating the dilation of the cavity, the hypokinesia of the septum and of the posterior wall, the closing anomalies of the valvular flaps.

Among the different clinical forms of congestive CMP the *idiopathic chronic congestive CMP* is the most frequent one, and is the congestive CMP for antonomasia. It affects more often young people or adults and its symptomatology varies in relation to the stage of the disease. The evolution towards congestive heart failure may be delayed even for years thanks to the strophanthin or digitalis therapy and to diuretics. This is in fact the only possible therapy as the causes of this disease are unknown.

The etiopathogenetic problem of the chronic congestive CMP is much discussed. The integrity of the coronary arteries, demonstrated in vivo by means of the coronarography and at autopsy, even as regards the smallest coronary rami, excludes the arteriosclerotic factor. The arterial hypertension can provoke a similar type of heart disease, and it may not be excluded that there exists in some cases a state of previously unobserved hypertension which may constitute a cofactor in the pathogenesis of the congestive CMP, as sustained by Brocking-

ton and Eddington (1972). On the other hand, any congestive CMP can during the phase of insufficiency present an arterial hypertension, especially diastolic, which then will be revolved with the return to compensation. This involves difficult problems of differential diagnosis as to the hypertensive cardiopathy, important also in connection of the therapy.

In certain cases there has been maintained the role of the chronic use of alcohol or unidentified causes of metabolic origin, in others the role of previous virus infections, especially by Coxsackie B and Echo virus, as suggested by Corsi and Sangiorgi (1963), Bengtsson (1968), Somerville (1968), Sainani *et al.* (1968) and by others. Kawai (1971) could demonstrate a higher incidence of antibodies fixing the complement with different viruses in the myocardium of subjects suffering from idiopathic CMP.

Whereas the pathogenetic role of virus infections remains uncertain in chronic forms, it assumes greater importance in *acute forms* which present the undoubted clinical picture of a true myocarditis, often with fever and positive humoral indices of infection (sedimentation rate, reactive protein C, etc.); but also in these cases the most careful virological researches remain often negative. The improved therapeutic possibilities with cortisones and cardiokinetics admit certainly in some cases to change from acute infective into a chronic congestive CMP, as I have had myself occasion to observe quite recently.

A nosographically well defined form of congestive CMP is the *peripartum CMP*, occurring before or soon after the partum. It is more frequently observed in women belonging to lower classes, especially in the negro race, often in twin pregnancy. This form occurs frequently also in our countries (Corsi *et al.*, 1968). It is believed that pregnancy would play a determinant role in predisposing the myocardium to the occasional action of extrinsic injurious, especially infective factors, through complicative moments of hemodynamic, neurogenic and endocrine order, typical of the more advanced stage of gestation and partum. In this connection it is interesting to mention that Farber and Glasgow (1970) have demonstrated that the encephalomyocarditis virus is capable of provoking myocardiac lesions more frequently in pregnant than in non pregnant mice.

Finally a short mentioning of the *tropical CMP*, of which a congestive form is known (quite similar to the chronic idiopathic congestive CMP in temperate countries), very frequent in South Africa and Nigeria; then a constrictive or mixed congestive-constrictive form, better known as African endocardiomyofibrosis, for the first time described by Davies in Uganda (1948). In England, Chew *et al.* (1977) described recently 11 cases of constrictive CMP, quite similar to the African form, in people who had never lived in Africa. This shows that endocardiomyofibrosis may occur also in temperate zones. In cases with re-

markable eosinophilia this form is indistinguishable from the parietal fibroplastic endocarditis, described by Löffler. So far it is not yet known whether the chronic congestive form of the tropical CMP and the endocardiomyofibrosis are the same disease, as it is believed by McKinney (1974), or two different diseases.

The form with constrictive imprint presents a reduced ventricular relaxation, an increase in telediastolic pressure, a ventricular tensiographic curve with dip-plateau and the acoustic finding of a "gallop rythm", moreover a clinical picture reminding the one of constrictive chronic pericarditis, but unlike this the ventricular telediastolic pressure is not alike on right and left, but higher on the left.

Also for these tropical CMP there have been invoked different, especially infective and malnutritional factors. Of particular interest are in this connection the experimental researches by Reid and Berjak (1966) and by Mc Kinney (1975); they would demonstrate the pathogenetic importance of the scarce alimentary intake of tryptophan, as it happens in several populations of Uganda that feed prevailingly on vegetables and nutriments rich in carbohydrates but poor in proteins.

Well, today it is believed that the congestive CMP are acquired CMP of multifactorial origin (infective, especially viral, dismetabolic, toxic, alimentary deficiencies). Their combination in a unique group may be justified by the uniform sequence of events which might lead to the terminal myocardiac damage. According to a hypothesis put forward by Laufer and Davies (1968), this damage would be caused by a disreactive mechanism, initially stimulated by the possible different noxae, which then, with the denaturation of the cellular elements would lead to the production of antimyocardiac autoantibodies and thus to the self-maintenance of the disease. Besides, it is possible that the different noxae damaging the myocardium prevent an adequate response of the RNA-DNA system, necessary for a good hypertrophic reaction of the myocardium. This explains the serious dilation and the often unfavourable and rapid evolution of these cardiopathies (Goodwin, 1974).

It can be believed that the group of congestive CMP will gradually diminish in the course of time as the progress of the diagnostic techniques will permit to ascertain with ever greater facility and certainty the causes that are everytime responsible for these cardiopathies.

Dystrophic cardiomyopathies

This group of true primary cardiomyopathies includes for the most congenital and/or familial cardiopathies, characterized by structural anomalies of the myofibres, probably due to a primitive disorder of development and growth

of the myocardiac tissue of unknown origin, maybe for metabolic genic alterations.

As I already mentioned in the introduction, we can make a distinction between forms that affect exclusively or prevailingly the contractile myocardium and those affecting exclusively or prevailingly the conducting system.

Among the main forms that affect the contractile myocardium there are the *hypertrophic forms*, the most frequent of these being the *obstructive hypertrophic CMP*, known also as idiopathic, hypertrophic subaortic stenosis (IHSS). Many cases of idiopathic cardiac hypertrophy or of familial cardiomegaly, described in the past, belong certainly to this form.

The anatomic-histological picture is characterized by the abnormal development in the different zones of the cardiac muscle, but especially of the deep bulbospiral muscle of the septum corresponding with the aortic ejection tract, of a particular tissue formed by monstruously hypertrophic myocells, immersed in an abundant ganga of sclerotic connective tissue and structurally desorganized in their architecture (as it has been well demonstrated at the electron microscope by Olsen (1971), Ferrans *et al.* (1972), Groszogaat and Battacharya (1972) because of anomalies of orientation, branching and cross-weaving of the myofilaments. The altered arrangement of the myofibrils would for some recall the characteristics of the embryonal myocardium, for others those of the rhabdomyoma. The characteristic macroscopic aspect is that of the so-called asymmetric hypertrophy, described by Teare in 1958. The value of 1.3 in the ratio between thickness of the septum and thickness of the posterior wall, as it was demonstrated by echocardiography, distinguishes the subjects with hypertrophic CMP from those with a normal secondary hypertrophy reactive to valvular aortic stenosis or to arterial hypertension (Henry *et al.*, 1973).

Ventriculography and echocardiography demonstrate well the reduced dimensions of the left ventricular cavity and the increased thickness of the septum and of the walls, often presenting a protuberance or "bulge" of the septum inside the cavity. According to our experience the myocardial scintigraphy with thallium 201 is also very useful in the diagnosis of these myocardio-pathies.

In these recent years it has been recognized that the ventricular obstruction is only one of the aspects, though not always present, of the hypertrophic CMP, as there are also *non obstructive forms* in which the hypertrophy develops not only in the septum but particularly in the free walls. The fundamental hemodynamic disorder which joins both the obstructive and the non-obstructive forms consists, however, in a loss of the normal distensibility, that is a reduced ventricular "compliance", due to the abnormal hypertrophy and sclerosis of the walls, which causes an increase in telediastolic ventricular pressure. This

is shown by an atrial gallop and an abnormally developed *a* wave in the apico-cardiogram and the carotid sphygmogram.

Cases with outflow obstruction present a systolic murmur of late onset, maximal at the apex and the center. Besides, the carotid sphygmogram shows some characteristic aspects, well described by Coblenz (1965), the most frequent being a quick ascension from hyperkinesia, then a mesosystolic depression of the plateau, corresponding with the maximal murmur during the phase of greater obstacle to ejection, followed by a second re-ascending which sometimes may hardly be denoted immediately before the diastolic incision. Such findings correspond clinically to a jerky arterial pulse and to the "pulsus bisferiens".

Besides, the anomalous contraction of the walls and of the papillary muscles provokes anomalies in the movement of the mitral valve, especially in the anterior flap. This explains the coexistence of a mitral regurgitation or of a simple mitral prolapse, provoking in such case a syndrome of the click and of the mesotlediastolic murmur of muscular origin, described in several familial cases by Froment *et al.* (1972), by Criley and Kissner (1976) and by Barlow and Pocock (1976).

There are various electro- and vectorcardiographic patterns, but the most typical are those of negative often very deep T waves (pseudo-ischemic type) or of abnormal Q waves (pseudo-necrotic type), by some authors attributed to activation anomalies of the septum, by others to confluent multiple sclerosis of the septum and the free walls. There may also be images of ventricular pre-excitation and intraventricular block.

A particularly interesting aspect, both from the physiopathologic viewpoint and the therapy of the heart disease, are the adrenergic influences on the loss of distensibility and the ventricular obstruction. Indeed the sympathetic stimulation determines a decrease in the ventricular compliance and an increase in the telediastolic pressure, whereas beta-adrenergic blockade provokes the contrary effect (Goodwin, 1974).

As to the ventricular obstruction, the beta stimulants (isoproterenol, adrenaline)—owing to their particular influence on the contractility of the deep bulbo-spiral muscle bundle of the aortic canal (Sciaccia *et al.*, 1972)—increase the obstacle to the ventricular emptying or make it appear when absent, thus increasing the intraventricular gradient between the inflow and the outflow tract of the ventricle and intensifying the murmur and the anomalies of the sphygmogram; a similar effect have digitalis, nitroderivates and muscular exercise. On the contrary, the beta blocking agents and substances stimulating alpha-receptors and increasing the peripheral resistances, like noradrenaline and methoxamine, diminish the intraventricular gradient and reduce the murmur. In connection with the variations of the contractility mention should be made also

of the phenomenon of remarkable diagnostic value (known as the Brockenbrough's sign) of the post-extrasystolic potentiation of the muscular stenosis.

The neurovegetative adrenergic influences, explaining the dynamics of the stenosis, let some authors believe that the role of the sympathetic system be of primary importance in the pathogenesis of the disease (Pearse, 1964; Condo-relli, 1974). Moreover, the association of subaortic stenosis with lentiginosis and neurofibromatosis (Polani and Moynihan, 1972; Goodwin, 1975; Elliott *et al.*, 1976), diseases due to a defect of the neural crest (the formation that controls the development of the sympathetic system), let someone believe that the cardiopathy be due to a primitive neuroectodermal defect. This hypothesis, however, is not generally accepted.

Besides the form that affects the left ventricle I might mention a rare hypertrophic CMP of the right ventricle, that affects prevalingly if not exclusively the septum from the right side (Dolara *et al.*, 1972); I described this as "idiopathic hypertrophic infundibular stenosis" (IHIS) (Sangiorgi *et al.*, 1974). Finally I may say that there are *forms for a long time asymptomatic* and non-obstructive which can present simple electrocardiographic alterations (Sangiorgi, 1974). The extension of the electrocardiographic and more recently of the echocardiographic study within families of subjects carriers of the cardiopathy enabled Van Dorp *et al.* (1976) to put in evidence that more than 30% may clinically seem asymptomatic; moreover, it was confirmed the concept that hypertrophic CMP represents a familial disease with dominant autosomal transmission of different degree of penetrance. Therefore echocardiography has today become of utmost importance as a non-invasive technique in the study of hypertrophic cardiomyopathies.

The more frequent symptoms are dyspnea, vertigos, syncopal attacks, whereas systemic and pulmonary thromboembolic complications are relatively less frequent. The symptoms appear at a mean age of about 28 years, and the mean survival after the beginning of the symptomatology is about 9 years with an annual mortality index of 3.5% (Hardarson *et al.*, 1973).

The prognosis is more connected with the ventricular telediastolic pressure than with the intraventricular gradient and would be more serious in subjects without obstruction because of the larger diffusion of the abnormal hypertrophy (Goddwin, 1974); this, however, by my own experience, does not always correspond with the reality.

These patients die for the most suddenly because of ventricular fibrillation, sometimes of cardiac arrest, in cases with ventricular blocks and other arrhythmias. In a reduced percentage of cases the cardiopathy evolves towards congestive failure, almost always after foregoing atrial fibrillation which is the most frequent arrhythmia; with the manifesting of insufficiency the signs of ventricular

obstruction disappear, establishing thus the hemodynamic picture of a hypertrophic-hypokinetic cardiomyopathy.

The most important aspect of the treatment is the use of beta-blockings because of their favourable effect, mainly on the compliance and less on the ventricular obstruction. Advisable are doses of 320 mg of propranolol or 800 mg of practolol per day; according to Hubner *et al.* (1972) these doses are generally well tolerated and seem to be efficacious in reducing the risk of sudden death. Only in cases with congestive heart failure it is advisable to use digitalis in association with the beta-blockings. Anyhow, the real efficacy of the beta blockings for improving the prognosis and the survival of these patients remains still to be established. The surgical treatment of myotomy of septal myomectomy has proved to be useful in single cases. Calcium antagonists are in study.

I shall not delay on the rare dystrophic CMP of sclerotic or sclero-atrophic type, as the so-called congenital papyraceous degeneration of the right ventricle or Uhl's disease, of which familial cases have not been described, and the right atrial idiopathic sclerosis with permanent atrial paralysis. It is interesting to remember that also diffuse familial fibrosis of the left ventricle with cardiac dilation and arrhythmias resembling the clinical picture of the sporadic congestive CMP, has been recently described (Ross *et al.*, 1978).

However, I would like to mention the much more frequent dystrophic CMP affecting prevalently the conducting system. The rare cases studied at autopsy put in evidence degenerative-sclerotic lesions of the conducting system and sometimes of the upper part of the intraventricular septum. It would therefore be a matter of a primary sclerotic disease of the His' bundle which ought to be called *Lenègre's disease*, the student who for the first time called attention to these forms with non-arteriosclerotic coronary pathogenesis.

In this group can be included different arrhythmias, for the most dromotropic, rarely bathmotropic, and electrocardiographic anomalies of familial character.

The most frequent form is constituted by *familial blocks* of juvenile or adult age (Sarachek and Leonard, 1972). In the younger generations will be found mono- or bifascicular branch blocks, in the older ones the disease evolves towards more serious forms of atrioventricular block. In these cases death occurs often suddenly because of ventricular fibrillation or cardiac arrest, which today can be prevented by pacemaker implantation. I have described a family where the larger part of the members presented a left anterior hemiblock (Sangiorgi *et al.*, 1974). Besides, there are families in which pictures of ventricular blocks or ventricular pre-excitation and isolated anomalies of the ventricular repolarization are associated (Hilmer, 1966; Sangiorgi *et al.*, 1970; Pellegrino, 1977). This shows that the alterations can affect not only the upper parts but also the

more peripheral ones of the conducting system at the level of the Purkynje fibres.

Finally there have been described familial cases of *bathmotropic arrhythmias*, especially ventricular tachicardia (by Waynberger, 1976) and junctional rhythms. I have described a most strange complex arrhythmia singularly similar in all of the family members, characterized by an allorhythmic combination of nodal rhythm and atrial and ventricular extrasystoles (Sangiori *et al.*, 1960).

A prospect of the better known clinical forms of primary CMP in young and adult age is reported in the table 2.

TABLE 2

Clinical forms of primary CMP (Sangiori, 1974, 1977)

-
- I. *Congestive CMP* (sometimes congestive-constrictive): apparently primary, probably secondary to multifactorial unknown noxae, non-familial
 - a) chronic congestive CMP;
 - b) acute congestive CMP (myocarditic form);
 - c) peri-partum CMP;
 - d) tropical CMP (congestive, constrictive type of endocardiomyofibrosis).
 - II. *Dystrophic CMP*: true primary cardiac myopathies, mostly congenital or familial
 - 1) of the contractile myocardium:
 - a) Hypertrophic CMP
 - obstructive:
 - left ventricle (idiopathic hypertrophic subaortic, IHSS);
 - right ventricle (idiopathic hypertrophic infundibular stenosis, IHIS);
 - non-obstructive;
 - asymptomatic;
 - possible muscular form of the mitral prolapse.
 - b) Sclero-atrophic CMP:
 - papiraceous degeneration of the right ventricle (Uhl's disease);
 - idiopathic right atrial sclerosis with atrial paralysis;
 - possible other forms (left ventricle, diffuse sclerosis).
 - 2) of the conducting system:
 - familial blocks;
 - familial bathmotropic arrhythmias;
 - preexcitation, familial anomalies of the ventricular repolarization.

In concluding this lecture on CMP of unknown etiology, it appears evident that, on the strength of the acquired notions, there can at present be established quite a satisfactory physiopathologic and clinical systematization of these cardiopathies, at least until further progress in the knowledges lead to new acquisitions on their etiopathogenesis, requesting to modify the proposed classification.

This nosographic arrangement of the primary cardiomyopathies, although it does not pretend to bring order in the chaos, as in vain tried by Hudson (1970),

it will at least attempt to contribute to a better understanding of this chapter of cardiology, still rather lacunous and controversial, but exactly for this reason extremely fascinating.

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Immunological aspects of kidney diseases and therapeutical implications

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At present two different immunological processes are supposed to be the cause of glomerular kidney diseases:

1. Production of antibodies;
2. Deposition of immune complexes in the kidney.

The pathogenesis of the former type is only found in about 5 per cent of the cases of glomerulonephritis, especially in the rapidly progressive and chronically progressive types and always in so-called Goodpasture's syndrome. The causes of such autoantibody production and the development of an autonomous process have not been uncovered completely. It is conceivable that exogenic factors such as infections or toxic hydrocarbons may trigger these autoaggression processes. If antibodies act against the basement membrane they are rapidly removed from the circulation due to the high affinity to kidney tissue. The antibodies permeate the pores of the endothelium cell and deposit within the glomerular basement membrane in a linear pattern. At the site of their reaction the antibodies also activate the complement system. By the chemotactic functions of C3a, C5a and the trimolecular C5/C7-complex leucocytes are attracted to the site of the antibody reaction. Having contact with the basement membranes that have been stripped of endothelium partly the leucocytes secrete enzymes destroying the tissue. The membrane structure is being destroyed. More leucocytes are allowed to approach and the inflammable process runs to pattern.

The causal importance of the antibodies to the triggering of the glomerular lesion results from the fact that it is possible to trigger such a glomerulonephritis by transferring these antibodies to monkeys. The detection of circulating antibodies is valuable. However, the detection of circulating antibodies requires highly sensitive techniques. The diagnosis is based upon the result of the kidney biopsy. The linear pattern of deposition of autolog immune globulines and of complement along the basement membrane is characteristic, but not absolutely pathognomonic.

The glomerular changes caused by immune complexes are by far more frequently found than the antibody-dependent types of glomerulo-nephritis. They

vary from the antibody-dependent types in the fact that neither the antigens involved nor the antibodies have primarily got anything to do with the kidney. The combination of the components is already effected within the circulation. While soluble immune complexes with a considerable excess of antigen or antibodies are as well harmless as large aggregates, which are rapidly eliminated by the reticuloendothelial system (RES), the immune complexes with only a slight excess of antigen show the property to deposit on endothelium. Smaller soluble immune complexes permeate the pores of the endothelium cells and the basement membrane. They accumulate on the subepithelial side of the basement membrane. Higher molecular but still soluble immune complexes, however, will not be capable of permeating the basement membrane. They accumulate in the mesangial space where they are phagocytized by the mesangium cells without causing a dysfunction of the glomerulus in the first place. When the mesangium cells have spent their capacity to phagocytize with constant supply of the highly molecular immune complexes from the circulation the immune complexes will be accumulated subendothelially. Then the thus deposited immune complexes activate the complement system with vasoactive substances likely facilitating the diffusion of the basement membrane.

Immune complexes are frequently formed in virus and bacterial infections, after vaccination, after transplantation and after medicamentous treatment. Unlike the frequency of the immune complexes a kidney lesion rarely occurs. With the lymphatic and the reticuloendothelial systems functioning normally the immune complexes that transitorily develop after antigen exposition are eliminated by the RES, and no immune complex nephritis will arise. A congenital or acquired disorder of the humoral response by which antibodies of low-grade quality and/or insufficient quantity are produced against one or several antigens both promotes the formation of relatively small soluble immune complexes with an excess of antigen and the antigen persistence which leads to an immune complex nephritis.

In addition to the humoral response, however, the cellular immune response is of importance, too. The coincidence between T-suppressor-cell-insufficiency and immune complex nephritis in natural and experimental animal models and at least in some types of the human immune complex nephritis is evident. This indicates to a genetically-dependent or postnatally acquired defect in the T-cell system. Thus in SLE-nephritis but also in other chronic types of glomerulonephritis the number and the function of the T-lymphocytes are decreased but those of the B-lymphocytes regularly increased. This leads to a violent immune complex formation. First the immune complexes are eliminated by the RES. Due to the excessive supply of immune complexes the RES capacity is exceeded, the immune complexes are circulating in the serum for a longer time

and are deposited intrarenally, which eventually leads to the activation of complement and biogenic amines and triggers complex nephritis. In addition to disorders in the area of the B and T-lymphocytes the insufficient phagocytosis capacity of the RES will likely be of importance as a third factor.

The diagnosis of the immune complex diseases is made by biopsy and subsequent immunohistological analysis. Granular deposits of immune globulines and complement on the glomerular basement membrane and/or within the mesangium are characteristic of immune complex glomerulonephritis. The granular deposits are mostly diffuse and generalized, rarely focally and segmentally arranged.

The aim of the therapy is clear. If nephritis results from chronic persistence of antigen in the presence of antibody capable of combining with it, but incapable of eliminating it, formed in an individual with mild, but important immunodeficiency, then several courses are open to us. As in the animal experimental work, in most situations in human beings where soluble complex nephritis was diagnosed, and the antigen was capable of elimination, the nephritis regressed; sometimes from a remarkable degree of uremia. The proportion of patients with nephritis in whom it is possible to identify antigens is far less than 1 per cent. From spontaneous nephritis in mice and other animals, it seems likely that the antigens involved in the bulk of human nephritis will be the common antigens to which all of us are exposed. Probably all of us form soluble complexes from time to time.

As only a few of us are taken ill with immune complex nephritis endogenic factors must be of importance which cause an atypical reaction of the macro-organism and thus will be important to the manifestation of an immune complex nephritis. The immune system is such an endogenic factor. From this follows another starting point for therapy: the effect on the immune balance. Our knowledge of the immune balance regulation is very poor. From a theoretical point of view an immune stimulation in various types of glomerulonephritis in certain stages to shift the antigen-antibody reaction in apathogenic areas or for overcoming the T-suppressor-cell-insufficiency would be useful. The stimulation of the antibody production and the cellular immunity, however, is still at the outset of its development. The results obtained by the few experiments with BCG, levamisol and the transfer factor were not very promising. At present the immune stimulation cannot properly be controlled, is insufficiently selective, and dangerous due to side effects.

Much experience has been gained with medicamentous immune suppression by means of corticosteroids, mustard-like drugs and purinantagonists. The conceptions that led to the introduction of the therapy some years ago have been partly superseded. According to current conceptions, an immunosuppres-

sive therapy only seems to be useful if it inhibits the function of the B-lymphocytes selectively. A B-cell hyperactivity only occurs in certain types that are accompanied by T-suppressor-cell-insufficiency such as in SLE-nephritis. In other forms of immune complex nephritis rather a reduced B-cell activity is supposed. It must also be taken into account that probably, with the given dosages, the antiphlogistic effect of the immune suppressiva may be more important than the immunosuppressive influence itself. In the clinic our initial hopes in the immunosuppressive therapy have not been fulfilled, because there are dangerous side effects in addition to the lacking or insufficient influence.

The application of immune suppressiva is viewed by the majority of authors as showing positive effects only in two groups of diseases, the SLE-nephritis and the minimal-change nephritis. While 20 years ago less than 10 per cent of the patients with SLE-nephritis have survived for two years, more than 50 per cent of the patients treated by corticosteroids have been kept alive for 12 years after the emergence of the clinical signs of a renal participation. In SLE-nephritis a highly dosed treatment by corticosteroid is recommended, which is gradually to be reduced after weeks. Frequently there will be an improvement of the renal findings only after 2 to 3 months. A combination of the corticosteroids with cytostatics is not supposed to be useful for the standard case.

Successes have been made also of the immunosuppressive treatment of the minimal-change lesion, a type of disease in which there are very few indications to the immune pathogenesis. Recently a pathological T-cell function has been discussed by the formation of a lymphokine that increases permeability. The best successes were observed in children in whom 95 to 100 per cent of the cases are considered steroid-sensitive and are highly susceptible to cyclophosphamid also with regard to the prolonged steroid-indurated remission. Thereby the steroid-sensibility closely correlates with the degree of selectivity of proteinuria.

In Goodpasture's syndrome and in the peracute proliferative glomerulonephritis the immunosuppressive treatment is considered inefficient. In recent years successes have been made with the combination of medicamentous immune suppression and exchange of plasma to rapidly remove the existing antibodies. Controlled trials that have been carried out for several years did not convincingly prove the success of immune suppressiva in the proliferative glomerulonephritis, the membranous nephropathy and in mesangiocapillary glomerulonephritis. In some trials the mortality even increased by treatment compared with a placebo group. There have been also objections to the execution and the evaluation of the controlled studies which mainly referred to the selection of the groups. To our mind a clinically controlled therapeutical experiment limited in time seems to be justified in single cases with clinical progression and the lack of a causal therapy such as removal of antigen.

The present state of the immune therapy of glomerular diseases is still unsatisfactory. For future treatment research should be concentrated on the following two key tasks:

1. Rapid and final elimination of infection agents by efficient virostatics and antibiotics;
2. Investigation into and influence on the differentiated control mechanisms of the immune response aimed at eliminating immunological noxae by antigen-specific tolerance induction, immune suppression or immune stimulation.

The pathogenesis and prevention of transient cerebral ischemia

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INTRODUCTION

The significance of thromboembolism to stroke

Recent studies on the incidence of the varieties of stroke have given a remarkably similar estimate of the importance of thromboembolism as contrasted with haemorrhage and other factors. In four contemporary studies, thrombosis and embolism accounted for 78-84% of *vascular stroke* (1, 2, 3, 4). The relative importance of thrombosis to embolism from cardiac sources varied, with embolism accounting for as few as 3% to as many as 19% of *strokes* in these four series. One of the reasons for the difference in the various series may be the care with which the heart is examined as a possible source of thromboembolism. Another is the fact that under the general heading of embolism, some would include cases which reflect embolism exclusively from cardiac sources while others would include those of arterial origin as well. Be that as it may, of greatest significance is the role of thrombogenesis in stroke production. As a result, all considerations of stroke prevention, for the time being at least must give emphasis to the possible benefits of anti-thrombotic therapy and the eradication of sources of emboli.

The mode of onset of thromboembolic stroke

A major calamitous stroke may be preceded by attacks of transient ischemia (TIA), by partial non-progressing stroke(s), or by an insiduously progressing stroke. Estimates of the number of strokes preceded by warning symptoms vary from as low as 10% (5) to as high as 79% (6). The majority of the estimates are in the higher range and give reason to the physician to be familiar with the warning symptoms and be prepared to consider their treatment.

The prognosis of TIA

The literature carries varying figures respecting the prognosis of threatened stroke. From the evidence available, it is reasonable to assume that there is

approximately a 5-6% chance per year of a stroke after TIA, with two-thirds of the deaths from myocardial infarction and less common vascular causes, and one third from stroke. Bright plaques in the retinal arterioles, one of the common findings with amaurosis fugax, may carry a worse prognosis. 15% of one large series were dead within the first year of the detection of Hollenhorst plaques (7).

THE PATHOGENESIS OF TIA

It is no longer possible to consider the symptoms of threatened stroke as if there were but one mechanism. Some earlier studies of TIA therapy, particularly those dealing with the possible value of anticoagulants, did not distinguish between these varieties. Since rational therapy will depend to a large extent on the origins of the symptoms, it is obligatory that this be given attention. Six main categories, as depicted in Table 1, account for these symptoms. Each category has a number of sub-groups which have been described elsewhere and will not be detailed here (8). From a practical viewpoint, the most important categories are the hemodynamic attacks, those of cardiac origin, those associated with lacunar infarction, and the artery-to-artery emboli.

TABLE 1

The pathogenesis of TIA

1. Hemodynamic factors	4. Other vasculopathies
2. Cardiac emboli	5. Lacunar infarction
3. Hypercoagulable states	6. Artery-to-artery emboli

Brief comment is in order about the recognition of TIA occurring despite occlusion of the artery appropriate to the symptoms. Several studies are now available which document the fact that an occluded internal carotid artery may still be associated with symptoms related to its ophthalmic, middle or anterior cerebral branches. The studies of Kendell and Marshall (9) make it appear improbable that these events might be expected commonly from hemodynamic mechanisms. On the other hand, recent studies (10, 11) suggest that there are some instances where ischemic events in the presence of occluded arteries may originate from orthostatic pressure changes in the cerebral circulation. Nevertheless, in the presence of an internal carotid artery occlusion, some embolic events originate from ulcerative and stenotic lesions in the external and common carotid arteries supplying the collateral retinal and hemisphere circulation (12). Indeed, recent evidence indicates that the internal carotid artery stump

itself, may be the site of atheroma and thrombus productive of embolic ischemic events (13).

THERAPY OF TIA'S

Brain infarcts are untreatable. Accordingly, attention must focus on prevention. The ultimate will be to prevent atheroma. In the interval, the prevention of stroke requires consideration of the following factors:

1. *Attention to risk factors*

It is outside the scope of this paper to elaborate in this area. Suffice it to say that the most rewarding risk factor to manage is hypertension.

2. *Lacunar infarction*

Most examples of lacunar infarction occur in significantly hypertensive individuals. The identification and proper control of hypertension are an obligation to prevent this disorder, and presumably to reduce or eliminate the possibility of its progression.

3. *Hemodynamic factors*

Table 2 lists the recognized and previously described hemodynamic causes of diffuse cerebral ischemia (14). For the most part, they produce diffuse disturbance of cerebral function and loss of consciousness. Because there is often

TABLE 2
Hemodynamic causes of diffuse cerebral ischemia

1. Orthostatic hypotension	5. Angina with cardiac decompensation
a) Spontaneous	6. Increased blood viscosity
b) Iatrogenic	7. "Steal" mechanisms
2. Carotid sinus hypersensitivity	8. Low perfusion states
3. Bradyarrhythmia—tachyarrhythmia	9. Vasospasm
4. Valvular aortic stenosis	10. Vascular compression by musculoskeletal structures

widespread bilateral disturbances with impaired awareness, they more closely mimic vertebral-basilar rather than carotid disease. In the first five varieties listed which relate to diffuse disturbance of cerebral circulation, focal features would be expected in a very small percent, possibly as low as 2%. The most important part of treatment is recognition of their existence. Valvular aortic stenosis is productive of ischemia both on a hemodynamic and on an embolic

basis. The other situations listed (6-10) are extremely uncommon, contribute a few cases to a large series and although important as isolated instances, are not to be given undue emphasis.

4. *Specific cardiac therapy*

To investigate by cerebral angiography and to recommend anti-thrombotic therapy for ischemic events that have a particular cardiac origin, such as bradarrhythmia, may be misdirected. Pacemakers, and at times, surgery, are the specific requirements. An unusual but recognized example of the latter is in the surgical removal of an akinetic or *aneurysmally* dilated segment developing as a sequel to an infarct and acting as a local area productive of thrombi.

5. *Specific treatment of vasculopathies*

Cerebral ischemic events occur with most of the rare and unusual disorders of the small arteries, including polyarteritis nodosa and granulomatous angiitis. Their recognition and specific treatment on these rare occasions are indicated.

6. *Vasodilators*

Convincing evidence that cerebral vasodilatation contributes to stroke prevention is completely lacking. Critical review of the literature and brochures on the subject would indicate that they are used on the basis of unfounded claims and uncritical analysis.

7. *Anticoagulants*

The major trials of anticoagulant therapy were carried out at a time when understanding of the pathogenesis of TIA, the significance of risk factors, and the design of clinical trials were all in the early stages of development and understanding. A recent exhaustive review of the whole subject has been carried out (14). On the basis of this review, Table 3 indicates the author's present conclusions respecting the use of anticoagulants in cerebral ischemic disease. The best evidence available is that relating to cerebral embolism from mitral stenosis with and without fibrillation, and from recent myocardial infarction. With ischemic events under these circumstances, the use of anticoagulants for a period of time would appear to be reasonably well-established. Similarly,

TABLE 3

Anticoagulants in cerebral ischemia

1. Mitral stenosis—Fibrillation	4. Hypercoagulable states
2. Recent myocardial infarction	5. Progressing stroke
3. Prosthetic heart valves	

the data indicates that anticoagulants are of value in patients in danger of embolization because of implantation of prosthetic heart valves.

Hypercoagulable states are difficult to define. Nevertheless, there is justification for consideration of anticoagulants in the management of ischemic events when the appropriate clinical circumstances are recognized, as in young women on the birth control pill. In these cases, what has been identified relates more to the coagulation than to the platelet aspects of thrombogenesis.

Progressing stroke presents a particular problem. Stroke in evolution does not necessarily reflect thrombosis in evolution. A worsening of a recent stroke may represent development of brain swelling or may represent changes due to secondary metabolic and hypoxic effects. At times, worsening that is varying in a stepwise progression over a number of hours, is a reflection of thrombus lying in one of the major arteries supplying the brain. There are those that feel that this is an indication for immediate surgery when it is detected by angiography. Others, including the author, believe that it is a hazardous surgical state and one that is best managed, at least initially, by anticoagulant therapy in the form of Heparin followed by Coumadin. Scientific accreditation of this therapy is wanting. It is not a sufficiently common situation that a reasonable trial has been or is likely to be conducted.

8. *Platelet-inhibiting drugs*

In 1965, Mustard (15) determined that a therapeutic agent, sulfipyrazone was capable of inhibiting some platelet functions including a reduction of platelet adhesiveness and a prolongation of platelet survival. Aspirin's platelet-inhibiting effect was discovered a year later. Interest in the possibility of using platelet-inhibiting drugs in stroke prevention was born. In November 1971, a Canadian collaborative trial involving methodologists and biostatisticians, hematologists and clinical neurologists was designed and was initiated using a double-blind randomized method and a factorial design to determine if patients with threatened stroke in carotid or vertebral-basilar territory would have fewer strokes and diminished mortality. Patients were stratified according to whether or not they had TIA or residual, and whether the attacks were in vertebral-basilar or carotid territory. All patients were required to have had ischemic events within three months of entry into the study. If they had nothing more than recurrent or single episodes involving symptoms as in Table 4, they were excluded from the study. Excluded also were patients who appeared to have cardiac or hemodynamic transient ischemic attacks, who had co-morbid conditions that might reasonably be expected to lead to their death within the five years of the follow-up, who were unable or refused to take the trial drugs, or who would require the trial drugs as contaminating therapy in the treatment of other disorders.

TABLE 4

Symptoms isolated or recurrent, not diagnostic of TIA

-
- | | |
|-------------------------|---------------------|
| 1. Diplopia | 4. Amnestic attacks |
| 2. Vertigo | 5. Drop attacks |
| 3. Unconscious episodes | |
-

The patients received 1300 mgms of aspirin in 4 divided doses with a sulfinpyrazone placebo, or 800 mgms of sulfinpyrazone in 4 divided doses with an ASA placebo, or both of these active agents, or neither. Thus, each patient received 4 capsules and 4 tablets a day. Rigid steps were followed to ensure compliance and lack of contamination.

24 cooperating hospitals in 12 neurological centres collaborated to enter 585 patients between November 1971 and June 30, 1976. The patients were then treated for one more year but no new cases entered the study. They were followed for an average of 1,003 days. During 92% of the time, patients were on the trial drugs. 99% follow-up was obtained and the results reported (16).

The analysis was a log-rank life-table analysis carried out in conformity with the factorial design recommended by Peto *et al.* (17). Analyzing all patients with the endpoints of TIA, stroke or death, there was a significant reduction by 19% in the aspirin-treated groups but not in those taking sulfinpyrazone or placebo. For stroke and death, there was a 31% reduction in the overall patient population. The sub-group of male patients analyzed for stroke and death had a 48% reduction and it was apparent that there was no benefit conferred upon female patients. It was considered that there was no statistical significance to any interaction between sulfinpyrazone and aspirin nor was there any synergistic effect demonstrated. Accordingly, the strength of a factorial design could be utilized and the 290 patients taking aspirin could be contrasted with the 295 taking sulfinpyrazone and/or placebo. Further sub-group analysis indicated that the best response in males occurred if they had not had previous myocardial infarction. In this particular sub-group 62% reduction of mortality and stroke rate was achieved (18).

The conclusion from this study was that platelet-inhibiting drugs have a place in stroke prevention. Aspirin is effective for males, but not for females and sulfinpyrazone was not effective in either males or females. Whether there is value in other platelet-inhibiting drugs or other combinations of platelet-inhibiting drugs is not yet known. A trial has currently been launched to determine if Persantine added to aspirin is more effective than aspirin alone.

9. Surgery

It is nearly 25 years since the first carotid endarterectomy was carried out for carotid stenosis. The question as to whether or not the incidence of stroke is reduced and stroke death diminished remains unsettled. One major joint study to test the value of carotid endarterectomy was carried out. A trend in favour of surgery for the group of patients who had ischemic events within the territory of the carotid artery productive of the symptoms, if the other arteries were in reasonably normal condition, was the best conclusion possible from this study (19). Many other reports are available which purport to indicate the value of the procedure. They compare the results with other dissimilar groups, or with some other natural history studies, or use patients submitted to carotid endarterectomy as their own controls. This is quite unsatisfactory. It is certain that a combined morbidity and mortality above 3% for the investigation and surgical procedure is not acceptable and institutions which cannot achieve this should best avoid the procedure. Carotid endarterectomy will have a limited place, at best, since estimates suggest that no more than 15-20% of victims of stroke have a surgically correctable lesion.

Of patients who have had carotid TIA's and partial strokes, 15% will have an occlusion of the cervical portion of the artery or intracranial carotid artery stenosis or middle cerebral artery stenosis. There is currently an enthusiasm for performing superficial temporal to middle cerebral artery bypass procedures in these circumstances. This procedure requires scientific appraisal before it can be accepted as standard therapy. This is now being carried out on an international scale, funded by the National Institutes of Health and involving countries of Europe, North America and Asia. Until such time as the study is complete, it would appear that this procedure should be regarded as interesting, innovative and unproven in stroke prevention.

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Neurohormonal factors in pathophysiology of the hypothalamic-pituitary system

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I. INTRODUCTION

The nature of the physiological interrelationships between hypothalamus and hypophysis has been only recently elucidated. The hypophisiotropic hypothalamus contains peptidergic neurons capable of synthesizing small peptides which stimulate or inhibit the synthesis and the release of pituitary trophic hormones. Hypothalamic hormone release is modulated by neurotransmitters elaborated in extra or intra-hypothalamic neurons. Neurohormonal activity has been demonstrated in hypothalamic extracts for the factors listed in table 1. Some of them have been isolated, identified and synthesized and are now available for clinical use. These discoveries have determined a tremendous expansion in our knowledge on the hypothalamic-pituitary function both in physiological and pathological conditions. This lecture will be devoted to the clinical aspects of these recent advances made possible by the efforts of many research groups and mainly by the pioneering basic work of Dr. R. Guillemin and of Dr. A.V. Schally.

II. NEUROHORMONES (*Review: Schally and Arimura, 1977*)

1. Thyrotropin-releasing hormone (TRH)

(*Review: Burger and Patel, 1977*)

A tripeptide [(pyro)Glu-His-Pro-NH₂] with thyrotropin releasing activity was isolated from ovine and porcine hypothalami and then synthesized.

1.1 *Diagnostic use of TRH*

TRH i.v. injection causes a prompt increase in serum TSH concentration that reaches the peak at 20-30 min. Values at 60 min are already significantly lower than those at 20 min while those at 120 min are still higher than the basal ones. An increase of about 50% in serum triiodothyronine (T₃) occurs at 120-180 min. In men, TSH response to TRH declines with age and is lower than

TABLE 1

Hypothalamic hormones and factors

<i>Hypothalamic hormone or factor</i>	<i>Abbreviation</i>	<i>Pituitary hormone controlled</i>	<i>Nature</i>
<i>Releasing hormones or factors</i>			
Thyrotropin-releasing hormone	TRH	TSH, (PRL)	Tripeptide (s)
Luteinizing hormone-releasing hormone	Gn-RH	LH, FSH	Decapeptide (s)
Corticotropin-releasing factor	CRF	ACTH	Dodecapeptide (?)
Melanocyte stimulating hormone-releasing hormone	MRF	MSH	Pentapeptide (?)
Growth hormone-releasing factor	GH-RF	GH	Decapeptide (?)
Prolactin-releasing factor	PRF	PRL	Tripeptide (?)
<i>Inhibiting hormones or factors</i>			
Growth hormone release-inhibiting hormone	GH-RIH Somatostatin	GH, (TSH)	Tetradecapeptide (s)
Melanocyte stimulating hormone-release-inhibiting factor	MIF	MSH	Tripeptide (s)
Prolactin inhibiting factor	PIF	PRL	?

(s) synthesized

in women. Minimal changes in thyroid hormone concentration, still in the physiological range, modify TSH response.

a) *Hyperthyroidism*. Owing to the inhibitory action exerted by thyroid hormones on thyrotrops, serum TSH response to TRH is abolished in untreated hyperthyroidism. This is the case in overt thyrotoxicosis, in subclinical cases, and in the exceptional cases of TSH hypersecretion-dependent hyperthyroidism. Absent responses can be observed in patients with autonomous thyroid adenoma (ATA) and in Graves'ophthalmopathy even in the presence of normal total thyroid hormone levels. However, increased serum free thyroid hormones (mainly FT_3) has been demonstrated in ATA (Beck-Peccoz *et al.*, 1978). Blunted TSH responses can be recorded in treated thyrotoxic patients several months after they have become euthyroid, probably because of previous long-standing inhibition of thyrotrops.

b) *Primary hypothyroidism*. In primary thyroid failure serum TSH is elevated and its response to TRH exaggerated. TRH test is useful particularly in sub-clinical cases with equivocal basal thyroid hormone and TSH levels.

c) *Central hypothyroidism*. We term central hypothyroidism thyroid failure secondary to hypothalamic-pituitary diseases. Absent or impaired TSH responses to TRH, suggesting pituitary insufficiency ("secondary hypothyroidism")

are found in about 30% of cases, while in the remaining patients positive TSH responses are seen suggesting a hypothalamic origin of hypothyroidism ("tertiary hypothyroidism"). Furthermore some patients with documented hypothyroidism due to hypothalamic-pituitary diseases have slightly elevated basal serum TSH, positive response to TRH (often exaggerated and/or prolonged) and poor or no increase in serum T_3 . In these cases the secretion of an immunoreactive TSH molecule with low or absent biological activity has been hypothesized and recently documented (Petersen *et al.*, 1978; Faglia *et al.*, 1979). Abnormal patterns of TSH response are frequently seen also in apparently euthyroid patients with hypothalamic-pituitary diseases.

d) *Hyperprolactinemic states*. Besides TSH, TRH is able to cause prolactin release. However, TRH does not seem to be the physiological regulator of PRL secretion. PRL response to TRH is usually absent in patients with PRL-secreting pituitary adenomas though a positive response cannot exclude the presence of a tumor.

e) *Acromegaly, Cushing's disease, gonadotropin-secreting pituitary tumor*. TRH administration causes growth hormone (GH) release in about 50-60% of patients with acromegaly. Although GH increases after TRH have been observed in several other pathological conditions (renal failure, liver cirrhosis, mental depression, anorexia nervosa, protein-calorie malnutrition) it has been suggested that GH response to a non-specific releasing hormone is due to receptor abnormalities of the adenomatous GH secreting cells in fact it occurs in *in vitro* fragments of adenomatous tissue from *in vivo* responsive patients (De Camilli *et al.*, 1978) and disappears after the complete excision of GH-secreting adenomas. This may have prognostic value in surgically treated acromegalics (Faglia *et al.*, 1978). ACTH release and LH in response to TRH have been described in Cushing's disease and in patients with gonadotropin-secreting pituitary adenoma respectively.

1.2. Therapeutic uses of TRH

So far TRH has no definite uses in therapy. An improvement in mental depression was described but not confirmed.

1.3. Analogs of TRH

(Review: Schally *et al.*, 1977)

Several analogs of TRH have been synthesized. 3-N-imidazole-methylhistidine-2-TRH and 3 (pyrazolyl-1)-Ala-2-TRH are more potent than the natural product in releasing TSH and PRL. Other analogs are equipotent with natural TRH but are more active on the CNS. This would justify clinical trials in mental depression.

2. Gonadotropin-releasing hormone (Gn-RH)

(Review: Mortimer, 1977)

This neurohormone was isolated from porcine hypothalami, structurally elucidated and synthesized. It is a decapeptide (pyro)Glu-His-Trp-Ser-Tyr-Gly-Leu-Arg-Pro-Gly-NH₂ able to stimulate LH and FSH synthesis and release.

2.1. Diagnostic use of Gn-RH

Gn-RH i.v. administration causes an elevation in serum LH and FSH levels that is usually greater for LH. Sex steroids modulate pituitary responsiveness to Gn-RH. The amplitude of the response is almost the same in adult men and in women during the early follicular phase of the menstrual cycle and increase throughout the menstrual cycle, the maximum being reached at midcycle.

a) *Primary hypogonadism.* In primary hypogonadism, both in women and in men, Gn-RH causes exaggerated gonadotropin increases. In normally virilized but oligo-azoospermic men, exaggerated FSH and normal LH increases can be seen because of the lack of inhibin.

b) *Hypogonadotropic hypogonadism.* Most patients with hypogonadotropic hypogonadism respond to Gn-RH. This suggests a hypothalamic rather than pituitary disease. However, a test based on a single injection of Gn-RH has been demonstrated to be insufficient to distinguish hypothalamic from pituitary hypogonadism. Thus, a sequential Gn-RH test has been developed: after several Gn-RH injections patients with hypothalamic diseases become responsive, while patients with pituitary lesions remain unresponsive or show a progressive decline in gonadotropin response. In prepubertal subjects, in anorexia nervosa and in hyperprolactinemic patients a preferential and sometimes exaggerated increase in FSH is often seen. This is not satisfactorily explained as yet, though probably mediated by altered ovarian steroid levels.

c) *Acromegaly and Cushing's disease.* GH and PRL in acromegaly and ACTH in Cushing's disease can be released after Gn-RH administration.

2.2. Therapeutic use of Gn-RH

a) *Male hypogonadotropic hypogonadism.* In prepubertal men with hypogonadotropic hypogonadism due to hypothalamic disorders Gn-RH administration (500 ug. s.c. every 8 hrs) provokes initiation and maintenance of puberty. In adult males with hypogonadotropic hypogonadism, including cases with idiopathic isolated FSH deficiency, an improvement of the sperm count can be obtained by Gn-RH treatment. Sexual potency can also be restored: this last effect is likely to be a behavioural effect of Gn-RH and seems independent of gonadotropin stimulation.

b) *Female hypogonadotropic hypogonadism.* In prepubertal women prolonged administration of Gn-RH (500 ug, 3 times a day) induces sexual maturation and menses. Follicular maturation and ovulation have been obtained by Gn-RH treatment. Gn-RH treatment is not indicated in infertile women in whom clomiphene administration can induce ovulation and in those with hyperprolactinemia in whom ovulation can be obtained by the removal of pituitary adenoma or by bromocriptine treatment. The best results are observed in patients with anorexia nervosa and in women with post-pill amenhorrea. In some cases additional HCG treatment is necessary to induce pregnancy. No multiple ovulations and births have been so far observed in Gn-RH treated women.

2.3. *Analogs of Gn-RH*

(Review: Schally *et al.*, 1977)

A number of structural analogs of Gn-RH have been synthesized. Some of them (particularly those substituted in position 6, 10, or both), are much more potent and longer acting than Gn-RH. Superactive analogs increase the possibility of the therapeutic use of Gn-RH. Some other analogs are devoid of Gn-RH activity and can compete for endogenous Gn-RH binding at pituitary level causing inhibition of gonadotropin secretion. Antagonistic analogs may be useful as a new birth control method and for treatment of precocious puberty.

3. *Growth hormone release-inhibiting hormone (GH-RIH, Somatostatin)*

(Review: Gomez Pan and Hall, 1977; Gerich and Patton, 1978)

A tetradecapeptide (H-Ala-Gly-Cys-Lys-Asp(NH₂)-Phe-Phe-Trp-Lys-Thr-Phe-Thr-Ser-Cys-OH) inhibiting the release of GH was isolated from ovine and porcine hypothalami and synthesized. This peptide was also found in high concentration in extracts of pancreas, stomach and duodenum. Somatostatin has a host of effects: only the effects on the pituitary will be considered. For information on the extrapituitary effects, see Gerich and Patton (1978).

3.1. *Effects on the pituitary*

a) *Growth hormone.* Somatostatin has a potent, though very short lasting, inhibitory effect on GH secretion. Somatostatin infusion blocks GH increases elicited by both physiological and pharmacological stimuli in normal subjects and in acromegalics. However, it is ineffective in blocking TRH and Gn-RH induced GH increases in acromegalic patients.

b) *Thyrotrophin.* Both basal and TRH-stimulated serum TSH are inhibited by somatostatin. This suggests a possible physiological role of somatostatin in controlling TSH secretion.

c) *Other pituitary hormones.* Suppression of PRL secretion in cultured rat pituitaries has been described but no definite effect has been observed on basal and stimulated PRL in normal man. Conflicting results have been reported on PRL secretion in acromegaly. Somatostatin has no effect on ACTH secretion in normal subjects, while it reduces plasma ACTH in patients with Nelson's syndrome and Addison's disease. Basal and Gn-RH-stimulated gonadotropin secretion is unaffected by somatostatin.

3.2. *Therapeutic use of somatostatin*

The very short lasting and multiple effects of somatostatin have prevented possible therapeutic use in diabetes and acromegaly. It is likely that in the future it will be possible to prepare long-acting analogs usable as therapeutic agents.

3.3 *Analogs of somatostatin*

(Review: Schally *et al.*, 1977)

In an attempt to obtain compounds with selective action and prolonged activity a number of analogs of somatostatin has been synthesized: Des-(Ala-Gly²)-GH-RIH, Des-(Ala¹-Gly²-Asn⁵)GH-RIH, and Des-Asn⁵-D-Trp-GH-RIH preferentially suppress insulin secretion; D-Cys¹⁴-GH-RIH and D-Trp⁸, D-Cys¹⁴-GH-RIH preferentially inhibit GH and glucagon secretion, D-Ala², D-Trp⁸-GH-RIH selectively inhibits GH release. Unfortunately none of them has prolonged activity.

4. Melanocyte stimulating hormone release-inhibiting hormone (MIF)

(Review: Kastin *et al.*, 1977)

MIF activity has been found in the hypothalamus of several species including man. Two peptides with this activity have been isolated and characterized. The most potent of them, L-Pro-L-Leu-Gly-NH₂ (MIF-I) does not seem to exert any definite endocrine effect in man. Some therapeutic activity in Parkinson disease and in mental depression has been reported.

III. NEUROTRANSMITTERS (Review: Müller *et al.*, 1977)

Neurotransmitters are the chemical signals ensuring communication between neurons and endocrine cells. In the hypothalamic-pituitary unit they act by influencing the activity of neurosecretory cells, though a direct effect on pituitary cells is also possible. The effects of Neuroactive agents capable of influencing central monoaminergic systems (mainly dopaminergic, noradrenergic and serotonergic) have been widely investigated in man. Though many aspects still

remain unclear, considerable progress has been made. The most important clinical implications will be briefly mentioned.

1. *Diagnostic use of neuroactive drugs*

Dopaminergic agents causes GH to rise in normal subjects, while they paradoxally inhibit GH secretion in about 60% of acromegalics. Thus, L-dopa can be used as a provocative test to assess GH secretory capacity in suspected GH deficiency, and piribedil has been shown valid to predict which acromegalic patients will be responsive to long term bromocriptine treatment. Dopaminergic drug-induced PRL decrease does not allow the discrimination between "tumoral" and "functional" hyperprolactinemia, however, nomifensine (a dopamine reuptake blocker) has been recently reported to achieve this end (Müller *et al.*, 1979). Anticatecholaminergic drugs are widely used as a provocative test for studying PRL secretion.

2. *Therapeutic use of neuroactive drugs*

Bromocriptine, a potent and long-acting dopaminergic drug, is extensively used for PRL suppression in lactating women and in hyperprolactinemic syndromes: by lowering serum PRL it restores ovulation and fertility in women and potency in men (Thorner, 1977). Evidence is accumulating that bromocriptine can reduce tumor growth in cases of PRL-secreting pituitary adenomas. Bromocriptine is a valid tool in the management of acromegaly (Chiodini *et al.*, 1975). Suppression of serum GH, reduction of soft tissues and improvement in glucose tolerance have been reported. Other dopaminergic agents such as lisuride and lergotrider have been found effective for the treatment of hyperprolactinemic and acromegalic patients. Methergoline, an antiserotonergic drug, has also been found capable of lowering both serum PRL and GH. Antiserotonergic drugs (methergoline, cyprohepatadine) have been reported to reduce ACTH secretion in Cushing's disease, but the results so far obtained are not univocal (Von Werder *et al.*, 1979).

IV. CONCLUSIONS

Neuroendocrinology has had a dramatic development in the past decade and is now moving into the clinical area. Although clinical investigations are still in an early stage, new syndromes have been described, many of those previously known better understood, new diagnostic procedures developed and new therapeutic perspectives opened.

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Current trends in the therapy of lymphomas

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Lymphomas are neoplasias originated at the lympho-reticular system which show a characteristic histological pattern, with an evident variability in their clinical symptoms, as well as in their pathological behaviour, therapeutical response and long-term prognosis.

These disorders represent different pathological entities, which include the Hodgkin's disease and no-Hodgkin's lymphomas. The clinical classification of lymphomatose patients, particularly the Hodgkin's disease, is based upon the anatomic extension of the disease.

It is important to define the type of lymphoma and to ascertain its clinical stage in order to give a suitable therapeutical orientation including the immunological profile in the complementary study. We do not perform neither laparatomies nor splenectomies, but in some cases we do perform laparoscopies. For the immunological profile we emphasize the necessity to investigate the membrane indicators, to apply a more specified chemio-immunotherapy according to the type of B or T cells. The distinction between lymphocytes B and lymphocytes T has both a diagnostic and prognostic value. In the future these studies will surely undergo new classifications. Control made in patients under treatment are useful to survey the clinical response. We have observed in Hodgkin that patients with a decreasing degree of sickness show a satisfactory response measured up by cells. Chemiotherapy constitutes the primary treatment for those stages considered as generalized.

Initially we employ chemiotherapeutic agents not associated with evident responses with a short-term margin of survival. For ten years we have been employing polychemiotherapeutic treatments and we have adopted five years ago the "MOPP", "MOP", "COPP" and "COP" protocols, slightly modified according to our personal experience and the availability of the necessary drugs. Different schemes were tried for the treatment of re-induction and maintenance. Already treated patients who had no response to the initial protocols were included in new plans with new agents. For three years on we have been making a contemporary treatment with BCG immuno-stimulants by scarification, levamisole, MER and *Corynebacterium Parvum*.

Medical problems in organ transplants

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The possibility of transplanting a healthy organ to substitute one which is chronically and irreversibly damaged by disease is now becoming successful clinical practice, at least for some carefully selected patients, and in the past 10 years more than 30,000 renal transplants and over 400 heart, liver and bone marrow transplants have been carried out (Table 1).

The problems common to the various types of transplant are: the choice of candidate, pre-transplant treatment, immunosuppressive therapy, and medical complications. In this paper renal transplants will be used as the point of reference, since most experience has been obtained in this type of transplant.

TABLE 1

Present status of clinical transplantation

	<i>Number of cases</i>	<i>Graft survival at 1 year</i>	<i>Maximum survival</i>
Kidney	>30,000	55-60%	>10 years
Heart	406	66%	> 5 years
Liver	418	40%	> 5 years
Marrow	430	50%	> 7 years

Choice of candidate

It is by now accepted that a transplant should be considered as an elective treatment, and precise clinical criteria must be established. In the last few years such criteria have been carefully followed, and results have improved. It has been estimated that about 20% of chronic uremic subjects may benefit from a kidney transplant (1), and it is probable that similar figures apply to the other types of transplant. This observation shows how we are still far from being able to consider transplantation as the treatment of choice for severe organ failure. The contributory limiting factors are usually classified as non-immunologic and immunologic.

Non-immunologic or clinical selection is important, and is connected especially with the mode of action and toxicity of the immunosuppressive treatment

regimens used today. With kidney transplants (but this is true also of all the other transplants) age is an important factor in the result, so that the percentage of graft survivals at one year is higher in the age group 15-45 years (1). Similarly, heart transplants are more successful in primary or congenital cardiopathies than in atherosclerotic cardiopathies (2, 3), and liver transplants are more successful in biliary atresia and Wilson's disease (which occur in younger subjects) than in other liver diseases (4). Another important factor contributing to graft survival is the degree of rehabilitation of the candidate (2): in fact, less suitable subjects have difficulty in overcoming the medical and surgical complications which occur frequently after kidney and other organ transplants. In addition, the clinical success of an organ transplant may be influenced by the type of disease which has damaged the organ to be transplanted; e.g. graft survival in transplanted, polycystic kidney patients is shorter than in other cases (5), but on the contrary in bone marrow grafts for aplastic anemia the results seem to be the same regardless of the etiology. Another way in which some diseases have a negative influence on the result of graft survival is that they may recur in the transplanted organ: this occurs with glomerulonephritis, lupus erythematosus and diabetes after renal transplants (5), coronary atherosclerosis after heart transplants (3), and, in particular, leukemia after bone marrow grafts and primary hepatic neoplasia after liver transplants (4).

As regards the immunologic factors, it is well known that these are of fundamental importance, though not always in the same way in the various types of transplant. In bone marrow grafts ABO compatibility and identical HLA and MLC are mandatory to avoid a graft-versus-host reaction. A close immunological match is also necessary in related kidney transplants. However, no correlation has been found between the degree of incompatibility and graft survival (rejection) in all the other cases (1). To explain such a dissimilarity, the possibility has been considered that the different loci in the HLA system may have varying degrees of importance; and recent research attributes particular significance to incompatibility at the Dr locus (6). In liver transplants an unexpected situation exists as regards immunological problems: here the incidence of rejection episodes is 10%, which is much lower than in other grafts. Furthermore, 10 liver transplants have been performed with ABO incompatibility. Lastly, reduced doses of immunosuppressive drugs are sufficient to keep the immunological response of the recipient under control (4).

In conclusion, the degree of immunogenicity in the organs and tissues considered is not identical, varying from a maximum in the bone marrow to a minimum in the liver, and from living to cadaver transplants; furthermore, the reasons for such differences, also observed in experimental animals, are not known.

Pre-transplant treatment

Prior immunization against a donor's antigens, which can be detected from the presence of cytotoxic antibodies in the circulation (crossmatch) is a contra-indication since it causes immediate rejection of the transplant. Conversely, the presence of circulating antibodies produced by prior immunization, but not specific for the transplant, has a more complex significance in that it is not always associated with an increased failure (rejection) rate. In fact, according to recent studies (7), there is a significant increase in the success rate of renal transplants when cold antibodies against B lymphocytes are present; however, such findings have not been confirmed by other authors (8). Kidney grafts are usually rejected when antibodies against HBAg are present. On the contrary, the prolonged presence of Au antigens in the blood, indicating a high degree of immunodeficiency, is usually associated with long transplant survival (1-5). Finally, there is usually a reduced incidence of rejection episodes in subjects who do not respond to DNB stimulation; however, their post-transplant mortality is greater since they are more vulnerable to infections because of their anergy (9).

There is another conditioning factor which is being given increasing importance: blood transfusions. In the past it was considered that these should be limited as much as possible in order not to immunize the patient and increase the risk of rejection; then, as far as cadaver transplanted subjects are concerned, it was noted that transfused recipients could be divided into two groups: those who form antibodies (responders) in whom the rejection incidence is higher than the average of the non-transfused subjects, and those who do not form antibodies (non-responders) in whom results are clearly better than average (10).

A thorough study of the effect of transfusions in kidney transplants in primates has shown that this effect not only facilitates the selection of transplant candidates (choosing non-responders), but also favors graft survival (11). Five transfusions some weeks before transplant significantly prolonged transplant survival of the primates treated in this way. Even a single transfusion prior to transplant prolonged transplant survival significantly. There was no difference between the effect of a transfusion of whole blood or one of erythrocytes only. However, better results were obtained when the donor's blood or erythrocytes were compatible with the recipient's antigens.

It is less certain that a transfusion performed on the day of the transplant itself will have a beneficial effect in man (12); there are conflicting reports on this point (13), but if it is proved that a beneficial effect is in fact produced by a transfusion performed on the day of transplant, this would make the policy of only carrying out transplants on transfused subjects much easier to apply.

The situation as regards survival of grafts from living donors is different.

For instance, in bone marrow grafts the rate of failures seems to increase in proportion to the number of blood transfusions given, and similar observations have been reported for living related kidney transplants.

Immunosuppressive therapy

The relatively high incidence of rejection in transplants in general, and GVHD in human bone marrow grafts, indicates that matching for the HLA chromosome by current methods is only a partial solution to these problems. It is therefore necessary to start immediately the immunosuppressive therapy that should be continued to maintain the graft survival. Apart from marrow grafts which require treatment different from other types of transplant, a combination of two or three drugs is usually used, namely, corticosteroids, cytostatics and ALG. By now, all the groups performing transplants use similar drug schedules; the schedule used for our series of 147 kidney cadaver transplants is shown in Table 2. Drug schedules similar to those used in kidney transplants are used in heart transplants, while in liver transplants the doses of azathioprine and corticosteroids are reduced.

The possibility, after a certain period of time, of continuing immunosuppressive treatment with a single drug has been evaluated in a limited number of

TABLE 2

Immunosuppressive therapy.

Kidney

Azathioprine 1-3 mg/kg/die

ALG 20-30 mg/kg \times 10-20 days or during rejection

Prednisone 100 mg/die \rightarrow 10-15 mg/die

3-5 pulse doses 10-20 mg/kg/first week or during rejection

150 rads \times 3 on the graft during rejection

Heart

Azathioprine, ALG and prednisone as in renal transplantation

Liver

Prednisone and ALG as in renal transplantation

Azathioprine 0.5-1 mg/kg/die

Bone marrow

Pretreatment: Azathioprine, corticosteroids, cytosine arabinoside, 6-thioguanine, daunorubicin, cyclophosphamide, TBI

Mitigation of GVH reaction:

Methotrexate 10-15 mg/m² i.v. on days 1, 3 and 6 and once a week thereafter

Prednisone 100 mg/die, if necessary

Fetal liver as source of HSC

cases. E.g., Lokkegaard and Thaysen suspended corticosteroid treatment in 40 subjects with kidney transplants which had functioned well for two years, and administered only azathioprine: no rejection crises were observed in 70% of the patients treated in this way (14). Sheriff *et al.* suspended azathioprine in 15 subjects with renal allografts after two years of treatment with combination therapy: a rejection episode occurred in only one case after prolonged suspension of azathioprine (15). Our series includes a woman with a four-year-old kidney cadaver allograft with low compatibility (only one antigen in common) who, unknown to us, stopped all immunosuppressive treatment six months after the transplant; 42 months after stopping the immunosuppressive the patient's renal function is excellent and she appears to be completely rehabilitated. Conversely, another two transplanted subjects spontaneously suspended immunosuppressive therapy, and less than a month later irreversible rejection crises occurred.

A new possibility for reducing graft rejection and GVHD is now being investigated in experimental animals (16, 17), namely cyclosporin A, a cyclic polypeptide extracted from two species of fungus, *Cylindrocarpum lucidum* and *Thricoderma polysporum*, with molecular weight of 1203, which has a powerful toxic action on proliferating T lymphocytes but not on resting lymphocytes. Even though we are dealing with preliminary data, it may be argued that the use of this substance in organ transplants in man may improve transplant results in the not too distant future.

Medical complications

These frequently occur, and are of different varieties depending on the primary disease which caused the organ failure, the artificial support treatment (e.g. extracorporeal dialysis), or the transplant itself. Only the complications connected with the allograft will be discussed, since these are common to all types of transplant. They consist of rejection phenomena and complications due to immunosuppressive treatment.

Obviously rejection crises are manifested in different ways according to the transplanted organ, and by a more or less acute reduction in their function. Specific immunologic criteria which allow this complication to be diagnosed in advance are not well-defined; therefore, rejection crises are mainly evidenced by clinical symptoms, and experience and readiness to take appropriate therapeutic measures are fundamental. Treatment consists of resuming the administration of ALG and giving high steroid bolus doses. There is no definite proof of the usefulness of other types of treatment, such as irradiation of the transplanted organ with 150 rads three to five times, or treatment with heparin and platelet antiaggregants. However, in our series all four types of therapy have been given concurrently, and a regression of acute rejection symptoms obtained

in 75% of the patients (18). On the contrary, no specific therapy is useful in the treatment of chronic rejection.

Another important group of possible medical complications consists of infections, which are the main causes of death of transplanted patients. Bacterial, viral or protozoal infections usually occur in the first few months after transplant, when immunosuppressive treatment is most aggressive. Post-transplant immunodeficiency has been the subject of several investigations, and it has been found that it is more evident in some organ transplants than in others, and that it is of major importance in bone marrow grafts. However, immunosuppressive treatment is probably not the only factor involved in causing immunodeficiency in the recipient. In addition, it is often difficult to identify the pathogens responsible for the individual infections since in many cases they are saprophytes with very little pathogenicity in a normal subject. In the attempt to prevent and overcome infectious complications several measures have been taken, none of which is effective by itself. These are: exclusion of subjects with chronic infections from the transplant program, pretransplant eradication of all potential infective foci, isolation of patients in protective environments during the most intensive periods of chemotherapy, prophylactic granulocyte transfusions as well as appropriate wide-spectrum antibiotics, suspension of cytostatic treatment in kidney transplants, and reduction of corticosteroid therapy. However, even if these measures reduce the incidence of infections, the latter still remain a significant cause of treatment failure.

As well as immunological and infectious complications, some metabolic disorders occur, for instance, steroidal diabetes, hyperadrenalism, hyperlipemia, and hypercholesterolemia, all of which need careful attention to avoid undesirable long-term effects.

Bone complications deserve some comments. They have been shown to occur particularly after kidney and liver transplants. Skeletal complications with clinical symptoms (aseptic necroses in the femoral head, spontaneous fractures, etc.) were demonstrated in 8% of our case series. However, bone involvement is more frequent than that. We have performed bone scintigraphy with ^{99m}Tc technetium diphosphonate and whole body scansion on 23 subjects with good renal function who had received a kidney transplant more than six months previously. Scintigraphic abnormalities were seen in 22 out of the 23 cases, and 20 of these subjects showed focal alterations in the cranium, ribs and femoral head. In the majority of cases no alterations in blood calcium, phosphorus and alkaline phosphatase were demonstrated. In addition, calcium kinetics, using ^{85}Sr strontium and whole body measurements, were studied. In the subjects with focal lesions the calcium exchangeable pool and accretion rate were at the lower limits of the normal, as in osteoporosis. The degree of the alterations observed

did not appear to be correlated with the age of the transplant, the amount of corticosteroid treatment, or the duration of dialysis treatment pre-transplant (19).

Another late complication following kidney transplants which has never been reported and which we have observed in 11 patients out of 147 transplants, is polycythemia with high levels of erythropoietin.

Finally, before concluding, I should like to touch on the rehabilitation possibilities which transplantation offers. A successful transplant produces the maximum degree of rehabilitation of the patient since it is a biological correction, albeit not always complete. No type of artificial correction, not even the sophisticated artificial organ systems which are used today, can give comparable results.

In conclusion, considerable progress in human organ and tissue transplantation has been made in recent years; however, major problems still remain. Answers to these will depend on increased knowledge of the biology of transplantation antigens, a more careful selection of recipients and donors using computer-based donor pools, further investigations of the role of immunodeficiency in the recipient, and, finally, improving immunosuppressive therapy.

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Toward a chronosemeiology and chronotherapy — cost-effective time — qualified reference intervals (prior to rhythm parameter estimations) for single (and serial) blood pressures, hormones or other determinations in internal medicine

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Medical applications of chronobiology (the study of biologic rhythms in the context of growth, development and aging) relate to 1) the quantification of healthy by *a*) chronodesms (time-qualified tolerance intervals) and by *b*) rhythm parameters — both *a*) and *b*) constituting more sensitive reference standards, as compared to conventional «normal values»; 2) the detection of *a*) earliest risk (recognized by rhythm parameter alteration) or of *b*) early (chrono)pathology; and 3) chronotherapy, the timing of treatment for optimizing known desired and minimizing known undesired effects and for investigating new pharmacodynamic effects upon rhythms — whether produced by old or new compounds.

For cost-effectiveness, chronodesms (*Experientia* 34, 713-716, 1978) can be established for peer groups of a given age and setting — at the outset. Individual chronodesms prove much more valuable. Even one or a few values for blood pressure or other variables of clinical interest can be rigorously interpreted chronobiologically, once chronodesms are available. Whenever single values repeated within a few hours and/or days fall outside the appropriate chronodesm, the collection of time series for a quantification of rhythm parameters on an individuals basis is indicated. In the case of certain hormones, such as plasma cortisol or prolactin, individuals chronodesms can be such that the same value is “too high” at one circadian time and/or “too low” at another, yet within the chronodesm at a third time. In the case of blood pressure, a series of self-measurements may preferably be followed by automatic indirect measurements for the evaluation of predictable spontaneous and/or reactive components of va-

riability. These evaluations can search for rhythm alterations, such as a change in one or several characteristics of a circadian (or other) rhythm best implemented by indirect monitoring at 10'-intervals (Halberg E. *et al.*, these proceedings) but documented as reference standard by the analysis of data obtained invasively.

A single cosinor analysis (*Physiol. Tchr.* 1, 1-11, 1972) of beat-by-beat measurements (Raftery, *Lancet*, Apr. 15, 1978, 976) revealed in "mesor-normotensive" patients a mean percentage rhythm (PR) of 87 and 91, with systolic and diastolic blood pressure mesors of 114 ± 1.4 and 68 ± 0.8 and mean double amplitude (measuring extent of predictable change) of 46 and 35 Torr, respectively. For mesor-hypertensive patients, mean PRs were 61 and 85%, mean systolic and diastolic mesors 159 ± 2.6 and 97 ± 1.2 and mean double amplitudes 42 and 36 Torr, respectively.

SYMPOSIA
METABOLIC AND BLOOD DISEASES
DUE TO ENZYME DEFICIENCY

Recent advances in hemolytic anemias due to enzyme deficiency

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General picture of enzyme defects of the erythrocyte

Hemolytic anemias can occur because of hereditary disorders of the human erythrocyte including, *a*) abnormal hemoglobins, *b*) alterations in membrane structure and function, *c*) enzyme deficiencies (Valentine and Tanaka, 1978).

Table 1 lists those enzyme defects which, to the best of our knowledge, are associated with hemolytic states according to a cause-and-effect relationship. It does not include other enzyme defects for which this relationship has not emerged with certainty (e.g., diphosphoglycerate mutase, enolase, glutathione peroxidase) nor those defects which produce clinical disturbances other than hemolysis [e.g. phosphoglucumutase and methemoglobin reductase(s)].

Although all of the enzyme defects listed in Table 1 are genetically transmitted, some of them can also occur as "acquired" abnormalities, i.e. as epiphenomena of various hematological disorders (Beutler, 1978b) or of inadequate supply of nutrients. The latter case is best exemplified by deficiencies of glutathione reductase, which are corrected by physiological amounts of riboflavin (Beutler, 1969).

TABLE 1

RBC Enzyme defects associated with hemolytic anemias

Enzyme	Type of defect		Hemolysis	
	Genetic	Acquired	Acute	Chronic
Hexokinase	+	—	—	+
Hexose phosphate Isomerase	+	+	—	+
Phosphofructokinase	+	+	—	+
Pyruvate Kinase	+	+	—	+
Glucose 6-phosphate Dehydrogenase	+	—	+	+
Glutathione Reductase	+	+	+	+
Pyrimidine 5'-Nucleotidase	+	+	—	+

Miscellaneous: Fructose 1,6-bisphosphate Aldolase, Triose phosphate Isomerase, Phosphoglycerate Kinase.

Another way of classifying the enzyme defects of the erythrocyte concerns the type of hemolysis they produce, either acute (triggered by a variety of exogenous agents) or chronic, or both. In the last case, which is typical of deficiency of Glucose 6-phosphate dehydrogenase (G6PD), different genetic variants of the enzyme are however responsible for acute or chronic hemolysis.

Glucose 6-phosphate dehydrogenase deficiency: metabolic consequences

By far, the most frequent among these enzyme defects is that of G6PD which has been estimated to affect, in its polymorphic genetic and clinical manifestations, approximately 100 million males throughout the world (Yoshida, 1973). This prevalence justifies the widely growing literature on G6PD deficiency which will therefore be considered here as a model of other enzyme defects of the human erythrocyte. The molecular pathology of G6PD deficiency will be especially considered here since other topics concerning this sex-linked enzyme protein have been extensively covered in several reviews (Bonsignore and De Flora, 1972; Kirkman, 1972; Fialkow, 1972; Yoshida, 1973; Piomelli, 1974; Luzzatto, 1973, 1974 and 1975; Siniscalco, 1974; Beutler, 1978*a* and *b*; Luzzatto and Testa, 1978).

Figure 1 illustrates the role of G6PD and of its partner enzyme 6-phosphogluconate dehydrogenase in the metabolism of human erythrocytes. Thus, the hexose monophosphate shunt, glutathione reductase and glutathione peroxidase represent an adequate defence of the cell against any oxidative challenge resulting

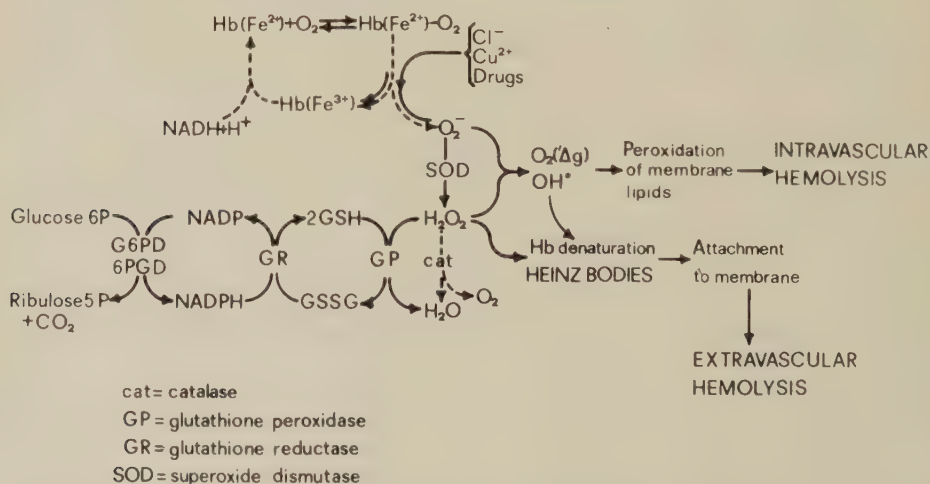


FIG. 1. Role of G6PD in the redox metabolism of human erythrocytes (from Luzzatto and Testa, 1978, modified).

in over-production of H_2O_2 and superoxide anion (O_2^-). The latter compound can be formed in the erythrocyte by several ways and especially through auto-oxidation of HbO_2 which generates also methemoglobin (Wallace and Caughey, 1975). Although auto-oxidation can be a spontaneous process, it is strongly accelerated by a variety of agents including some potentially hemolyzing drugs such as antimalarials, sulphonamides, certain antibiotics, etc. The right part of the scheme, derived from a recent review by Luzzatto and Testa (1978), shows a tentative scheme for the role of O_2 in initiating the events culminating in hemolysis. Therefore, as a whole, we can understand how a genetically determined G6PD deficiency will affect the metabolic basis for biological defence against oxygen toxicity.

Glucose 6-phosphate dehydrogenase deficiency: molecular pathology

Three general mechanisms can underlie any enzyme deficiency (Luzzatto, 1974): *a*) synthesis of a mutant enzyme protein associated with low catalytic efficiency, *b*) synthesis of a functionally normal enzyme protein being degraded more rapidly within the cell, *c*) decreased synthesis (because of a regulatory or structural mutation) of the enzyme protein. Moreover, combined mechanisms are also possible.

Mechanism *a* can be estimated by measuring the enzyme specific activity, i.e. the ratio of catalytic activity to the concentration of that enzyme protein, in crude cell lysates. Availability of a procedure for G6PD purification from pooled human erythrocytes (De Flora *et al.*, 1975; Morelli and De Flora, 1977) was exploited to raise anti-G6PD antisera and to develop a specific radioimmunoassay (RIA) for G6PD (De Flora *et al.*, 1978). This allowed the specific activity of this enzyme protein to be measured in hemolysates from normal individuals (G6PD B) and from G6PD-deficient subjects. A check for these estimations was made on G6PD preparations highly purified from erythrocytes of single donors by means of a simplified procedure based on affinity chromatography (Morelli *et al.*, 1978). These techniques showed that the specific activity of G6PD B is 180 I.U./mg (of G6PD protein), while that of G6PD Mediterranean (the polymorphic variant usually found in Sardinia, characterized by a severe deficiency in activity) is 69 I.U./mg and that of G6PD Seattle-like (a rare variant) is 151 I.U./mg.

A further issue was the estimation of G6PD specific activity in erythrocyte fractions of different age, obtained by centrifugation on discontinuous density gradients (Corash *et al.*, 1968). The results showed a consistently accelerated intracellular breakdown of the two G6PD-deficient variants *versus* the B type, a good correlation holding between the rate of decay and the severity of the defect (Morelli *et al.*, 1978). Moreover, the patterns of decay during the life-

span of the relevant erythrocytes suggest that the rate of G6PD synthesis in the bone marrow is not impaired.

Table 2 summarizes the conclusions of this study. In the two G6PD variants tested the rate of intracellular breakdown is a crucial mechanism accounting for the defect of catalytic activity.

TABLE 2

Biochemical mechanisms of G6PD deficiency

<i>G6PD phenotype</i>	<i>Synthesis</i>	<i>Breakdown</i>	<i>Catalytic efficiency</i>
B	Normal	Normal	Normal
Mediterranean	Normal	Greatly enhanced	Impaired
Seattle-like	Normal	Enhanced	Slightly impaired

Glucose 6-phosphate dehydrogenase deficiency and proteolytic systems

The above results raise two questions: *a*) Which is the basis for G6PD deficiency in other polymorphic variants, such as for instance the A- variant which is very common in the U.S.A. and in Africa and is responsible for several acute hemolytic episodes? *b*) Which are the mechanisms whereby the intracellular breakdown of normal and mutant G6PD takes place?

In order to answer the latter question, we started an investigation on the proteolytic systems of the circulating erythrocyte, which are largely unknown, trying to correlate them with the breakdown of G6PD. The first indications we have obtained are consistent for a role of the erythrocyte membrane in the intracellular degradation of G6PD. In fact, *a*) the cytosol appears to lack G6PD-directed proteases, *b*) three distinct endopeptidases possibly acting on G6PD are associated with the membrane fraction (Pontremoli *et al.*, in preparation), *c*) some low but significant levels of G6PD activity are found on the hemoglobin-free membrane (Benatti *et al.*, 1978), and *d*) a marked interaction exists between purified G6PD (B type) and the cytoplasmic surface of membranes.

These data appear to support the view that cytosolic G6PD has an unequivocal affinity for the erythrocyte membrane which on the other hand seems to contain proteolytic activities competent for degradation of G6PD itself. The full characterization of these endopeptidases and their correlation with the intracellular breakdown of normal and mutant G6PD are the immediate aims of our present investigation. The long-term goal of this study is to ascertain the possibility of identifying some specific inhibitors of G6PD degradation in order to try to block the phenotypic expression of the genetic defect.

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Porphyrias due to congenital enzyme deficiency

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Porphyrias (P) were first defined and subdivided by Günther in his pioneer paper 1911. Now a system based on clinical, biochemical and hereditary features exists. P comprise a variety of diseases, and to diagnose P without specification is misleading. P are divided into 1) toxic (sulphonal, C_6Cl_6 Pb etc.), 2) erythropoietic, including the extremely rare congenital (CEP) with recessive inheritance and the more common erythropoietic protoporphyria (EPP) with dominant inheritance, and 3) hepatic, including the most common P cutanea tarda (PCT; chronic or symptomatic P) without simple Mendelian inheritance, and the acute porphyrias: acute intermittent (AIP), variegate (VP or mixed P) and hereditary coproporphyria (HCP). All P show many asymptomatic carriers, and the distinction hepatic—erythropoietic is not complete, as EPP and even CEP (Rimington and With, 1973) may show hepatic involvement, and the enzyme deficiency in AIP is found in both liver cells, fibroblasts and erythrocytes (Meyer *et al.*, 1972).

The most dangerous P are the acute hepatic showing porphyric attacks (PA). The division into AIP, VP and HCP is not sharp and it is likely that every family has its specific gene (With, 1969b). PA was first described by Danish psychiatrists (Fehr, 1891; Geill, 1891; With, 1971) who coined the triad: Pain, pareses and pink or dark urine (Friedenreich, 1892). The pain is due to sympathetic nervous lesions and is most often acute abdominal, but may be atypical and chronic (With, 1977). The pareses often mimic polyneuritis, are transitory, but may be permanent (Sørensen & With, 1971). Acute psychosis, epileptic attacks and amblyopia occur. The urine is rich in delta-aminolevulinic acid (ALA) and porphobilinogen (PBG) and will always show a positive Hoesch test during the acute phase (Lamon *et al.*, 1974). PBG is converted to porphyrin in acid urine for which the test may be negative if the urine is not fresh, but if so screening for porphyrins is usually positive.

The pathogenesis of the PA is unknown, but it may be due to the extremely high concentrations of ALA and PBG present at the height of the attack (Eales *et al.*, 1971). PA is usually precipitated by drugs, especially barbiturates, but they act irregularly (With, 1969b; Eales, 1971). To avoid attacks early diagnosis

of PA and detection of carriers is important. To do this one must have the possibility of PA in mind and perform family investigations in every new case of AIP, VP and HCP. The diagnosis of acute PA is easy with the Hoesch test which is simple, specific and cheap. If it is negative in fresh urine the symptoms in question cannot be due to a fresh PA. For family studies more refined measures are required and the same is the case to distinguish the different forms of P (With, 1978). Here analysis of feces is necessary. Treatment of PA is largely symptomatic—as artificial respiration and physical therapy—and the only measures proposed to counteract the metabolic basis is carbohydrate rich diet and infusions of heme acting by end product repression of the biosynthetic chain (Watson and Pierach, 1978). With these measures fatal attacks of PA are rare.

Cutaneous P is most often PCT, with normal excretion of ALA and PBG, less often VP where the excretion of ALA and PBG and fecal porphyrins are often increased and where PA occur in patient and relatives, more seldom HCP where the fecal coproporphyrin is highly increased, and extremely seldom CEP, beginning already in childhood. To this comes EPP—also seen in childhood—which is relatively common and shows normal urinary porphyrins but increased protoporphyrin in the erythrocytes. If urinary porphyrins are normal in a patient suspected for cutaneous P it is therefore important to measure erythrocyte porphyrins, and if this is normal also plasma porphyrin (With, 1978).

The biochemical basis of P is various enzyme deficiencies which are now beginning to come to light, but our knowledge is still incomplete for which diagnosis of P must at present be based on the clinical picture, the biochemical pattern of excreta and blood, and the heredity. The first enzyme anomaly detected in a human patient with P was an increase in the rate-limiting enzyme of the heme biosynthesis chain delta-aminolevulinic acid synthetase (ALA-S) in the liver cells by Tshudy *et al.*, (1965), who used modifications of the methods of Granick and Urata (1963) and confirmed their results found in experimental porphyria in animals. Later it was discovered that the primary enzymatic defect was a decrease of uroporphyrinogen synthetase (UPG-S) which gave rise to increased ALA-S by end-product repression, and this defect was found not in liver cells only but in erythrocytes too, and both in patients with AIP and latent carriers (Meyer *et al.*, 1972). This finding was later confirmed by a number of investigators (Kramer-Birnbaum and Tomio, 1976; Astrup, 1978; Doss and Tiepermann, 1978; Formgren and Wetterberg, 1978) including myself (not published). After the description of a simple and relatively cheap technique for determination of erythrocyte UPG-S which is a stable enzyme (Peters *et al.*, 1976) it can be used in clinical chemistry to disclose carriers of AIP and is the only method to detect AIP-carriers before puberty. In the few cases VP and HCP studied the erythrocyte UPG-S has been found normal. There is a not

inconsiderable overlap between the UPG-S levels in normals and AIP-carriers; thus we found—in nmol/ml/h—in 45 normals 8-45 (all except 3 above 15) and in 29 carriers of AIP 4-17 (all except 4 were 15 or below. We also studied erythrocyte UPG-S in 27 normal newborn to investigate the possibility to detect AIP-carriers at birth, and found 25-80 (13 above 45), i.e. a higher level than in adults. We also found that practically all the enzyme activity disappeared by heating the blood 30 min to 60° while in adults only a minor loss of activity follows such treatment.

Enzyme studies in other forms of P are more scanty. Decreased coproporphyrinogen oxidase has been found in HCP (Brodie *et al.*, 1977; Grandchamp and Nordmann, 1977) in VP moderately reduced ferrochelatase has been found in the bone marrow (Kramer *et al.*, 1977) and in EPP a marked decrease of this enzyme has been demonstrated in the erythrocytes (Kramer *et al.*, 1977). In PCT an enzyme block at the UPG-decarboxylase level has been demonstrated. In CEP decreased UPG-isomerase (UPG-cosynthase) has been found (Levin, 1968) and Alcira Batlle and collaborators (not published) found greatly increased ALA-S and UPG-S in lyophilized bone marrow from my porphyric bull (Rimington and With, 1973).

These enzyme studies are interesting, and can to some degree explain the excretion patterns in the different forms of P by assuming that the enzymatic blocks cause secondary increase of ALA-S by end product repression mechanism, but much is unexplained. Enzyme analyses in P are still within the realm of research except erythrocyte UPG-S which is today a valuable tool in the clinical chemistry of AIP.

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Familial lecithin: Cholesterol acyltransferase (LCAT) deficiency

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Familial LCAT deficiency was first described in 1967 in three Norwegian sisters (1, 2). Today 21 patients from 10 different families have been described. Four of the families come from Scandinavia (1, 3, 4, 5) (Table 1). The others have been detected during later years and come from all parts of the world (6, 7, 8, 9, 10, 11) (Table 2).

TABLE 1

Familial LCAT deficiency - Scandinavian families

<i>Fam</i>	<i>Patients</i>	<i>Sex</i>	<i>Born</i>	<i>Corneal opacity noted</i>	<i>Protein- uria from</i>	<i>Anemia detected</i>	<i>General health</i>
I	A.R.	F	1933	appr. 1948	1952	1952	1969 renal failure Transplant. Jan. 1973. Died July 1973.
	I.S.	F	1935	at puberty	1954	1954	Kidney transplantation 1977
	M.R.	F	1947	appr. 1962	1964	1965	healthy
II	M.L.	F	1921	in childhood	1925	1965	Died in uremia 1976
	B.B.	M	1924	1958	1941	1961	Died in uremia 1964
III	A.A.	F	1926	1966	1958	1958	Healthy
	L.G.	M	1932	1962	1950	1962	Kidney transplantation 1976
IV	K.Å.	M	1918	in childhood	1943	1956	Kidney transplantation 1973
	D.J.	F	1913	in childhood	not present	in childhood	Healthy

The *fundamental defect* is the lack of functioning LCAT in plasma. This enzyme, synthesized in the liver normally catalyzes the transfer of a fatty acid from lecithin to free cholesterol (Fig. 1). Plasma lipids are characterized by marked decrease in the concentration of cholesterol esters and lysolecithin and increase in free cholesterol and lecithin.

TABLE 2

Known patients with familial LCAT deficiency - may 1978

Patient	Sex	Born	Age at diagnosis (years)	National origin	Corneal Opacity	Anaemia	Procuria	Uremia	Cholesterol total MG%	CE %	TG MG%
1	F	1936	34	Norwegian	+	+	+	+	300	3	400
2	F	1934	32	Norwegian	+	+	+	+	500	8	570
3	F	1946	20	Norwegian	+	+	+	—	140	5	130
4	F	1921	48	Swedish	+	+	+	+	369	27	533
5	M	1935	—	Swedish	+	+	+	+	137	?	?
6	F	1926	44	Norwegian	+	+	+	—	215	14	900
7	M	1932	38	Norwegian	+	+	+	+	235	13	630
8	M	1918	55	Norwegian	+	+	+	+	133	3	251
9	F	1913	60	Norwegian	+	+	—	—	107	2	105
10	M	1942	30	Italian	+	+	+	—	131	13	190
11	M	1942	28	Italian	+	+	+	—	64	31	169
12	M	1955	20	East Indian	+	—	—	—	83	10	107
13	M	1945	30	Engl.-Canadian	+	+	?	—	175	12	?
14	M	1940	35	Engl.-Canadian	+	+	+	—	100	13	?
15	F	?	—	French	+	+	?	—	175	8	250
16	F	?	—	French	+	+	?	—	?	?	?
17	M	1959	16	Ital.-Swedish	+	+	+	—	162	3	340
18	F	1954	21	Ital.-Swedish	+	+	min	—	120	25	278
19	F	1946	30	Japanese	+	+	min	—	42	0	110
20	M	1948	28	Japanese	+	+	+	—	94	20	222
21	M	1950	26	Japanese	+	+	+	—	163	29	346

The clinical characteristics of the disease are: Corneal opacities in all layers of the corneal stroma with marked lipid arcus occurring at young age (Fig. 2), target cells in peripheral blood smear, foam cells in bone marrow and in kidney glomeruli (Fig. 3) as well as Sea-blue histiocytes in spleen and bone marrow. Anaemia and proteinuria occur in most patients and early in life. Plasma is often milky or turbid. The life threatening complication is the development of renal insufficiency that may occur rapidly and without warning in adult life.

Active LCAT has not been detected since injection of radioactive mevalonate did not lead to label plasma cholesteryl esters. Such esters did, however, become label in the patient after peroral intake of radioactive cholesterol, suggesting that the small amounts of cholesteryl ester present in plasma is formed in the intestine.

Individual plasma lipoprotein fractions are all abnormal. High density lipoproteins (HDL) are heterogenous. Large HDL's have EM-appearance of stacked disks like those seen in liver disease. Low density lipoproteins (LDL) are also heterogenous. A large molecular weight fraction (LM-LDL) appears as

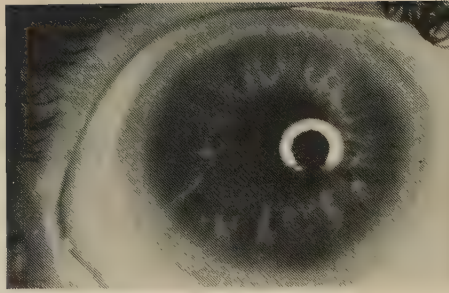


FIG. 1. Principal lipid reactants in the plasma lecithin: cholesterol acyltransferase reaction.

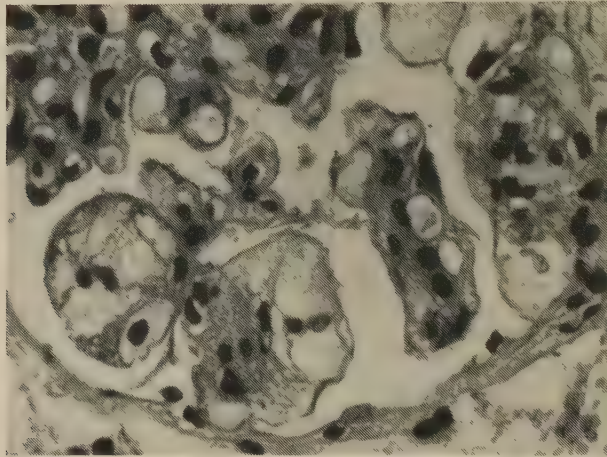


FIG. 2. Corneal opacities most prominent in the periphery.

LECITHIN:CHOLESTEROL ACYLTRANSFERASE

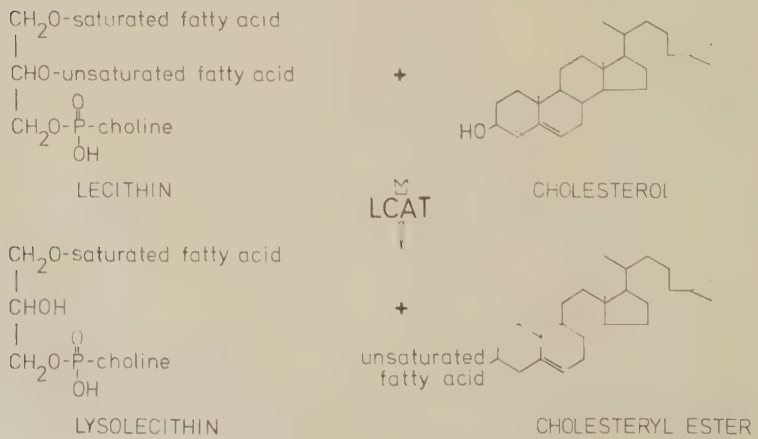


FIG. 3. Foam cells in a glomerular tuft.

large flattened structures up to 1 000 Å in size. Intermediate size LDL'S similar to the LP-X of cholestasis is regularly found. Also small LDL's with normal appearance, but high content of triglycerides are found (12).

Erythrocytes are abnormal with increase in cholesterol and lecithin, but decreased amounts of phosphatidyl ethanolamine and sphingomyelin. The erythrocytes lipid abnormalities are reversible.

In the *kidneys* lipids are deposited in the basal membrane that is of irregular thickness with fenestrated and abnormal endothelium. *Early atherosclerotic* changes are characteristic for the disease.

One clinical sub-group has been detected characterized by the lack of proteinuria and renal insufficiency. In this sub-group LM-LDL has not been detected.

Genetical studies suggest autosomal recessive mode of inheritance. Genetical statistical methods have proved that genetical linkage between the haptoglobin gene and the LCAT gene is present. The *LCAT gene* is located in close proximity to the alpha-haptoglobin locus situated between the middle and terminal point of the *long arm of chromosome 16*. This is the first linkage group to be reported on this chromosome (13).

In *therapy* a diet restricted in fat is advised because it reduces the mounts of LM-LDL. Plasma transfusions reverse the abnormal biochemical findings in plasma and erythrocytes, but should be avoided because many patients may have to be transplanted.

Of the nine Scandinavian patients two have died in uremia unoperated, one died half a year after kidney transplantation. Three others live with transplanted kidneys. Kidney biopsy after transplantation show principally the same changes with lipid deposits before operation showing that the renal disease is secondary to the plasma LCAT deficiency and lipoprotein abnormalities.

Familial LCAT deficiency is most likely a single genetic defect caused by the lack of LCAT activity in plasma. The variations observed in biochemical and clinical expression must be explained by the normal variation in genetic constitution and external factors.

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Drug toxicity due to enzyme deficiency

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Toxic side effects due to drugs do not only follow overdosage of pharmacological therapy. Differences in effects as well as side effects of a given pharmacon can also be dependent upon the genetic variability of enzymes which govern metabolic processes of the drug. Quite a number of such examples have been demonstrated within the last 15 years. The science analysing this connection has been coined "Pharmacogenetics" (15, 22). Genetically coded differences in the protein structure of the same enzyme can be of qualitative or quantitative nature. Within one defined enzyme group the number of variants can be a small one so that they do not show up after statistical analysis on a unimodal distribution curve.

Within large groups however the distribution can reflect an existing polymorphism by means of bi- or trimodal curves of enzyme activities. The character of distribution curves on the other hand is influenced considerably by the experimental method employed.

In this overview not so much the genetical basis, but rather the biochemistry and clinical importance of existing enzyme variants shall be elucidated. As examples of toxic drug side effects in connection with enzyme variability shall be discussed: Firstly the polymorphism of INH-acetylation, secondly pseudocholinesterase deficiency as the cause of prolonged apnoea after succinylcholine application, thirdly the disturbance of diphenylhydantoin hydroxylation and as a fourth point the intolerance to acetophenetidin.

1. The polymorphism of INH-acetylation

Mitchell *et al.* (14), Knight *et al.* (13) as well as Evans *et al.* (2, 3) have demonstrated that the concentrations of isoniazide in a larger group of people were distributed in a bimodal fashion after application of 4 or 10 mg INH/kg (Figure 1). INH is excreted in the urine as acetyl-INH, isonicotinic acid, INH as well as in other metabolic forms. According to Goedde *et al.* (7) in the monkey the activities of liver-N-acetyltransferase, which metabolises INH in the presence of Coenzyme A into acetyl-INH, are in reverse proportionality to the INH concentration in the serum. In case of a high rate of acetylation the INH concentrations are low. The reverse is true for a low rate of acetylation.

Depending on the height of INH concentration in the serum “rapidly acetylating” individuals (Ac^R/Ac^R) are discriminated from “slowly acetylating” individuals (Ac^S/Ac^S). According to formal genetics one is thus dealing with a two-allele-model for N-acetyltransferases encompassing three genotypes (Ac^R/Ac^R , Ac^R/Ac^S , and Ac^S/Ac^S).

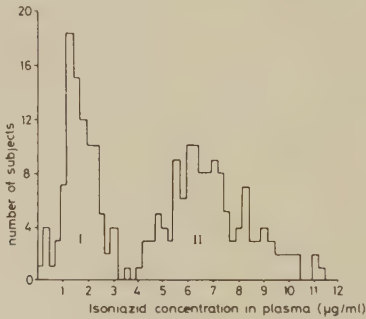


FIG. 1. Bimodal distribution of plasma-isoniazid concentration in 267 persons after ingestion of 10 mg isoniazid per kg body weight. Evans *et al.*, 1960 (2).
I Ac^R/Ac^R , Ac^R/Ac^S
II Ac^S/Ac^S

Studies employing population genetics resulted in different gene frequencies for different parts of this world as shown in table 1. Whereas in average the number of “slow acetylators” is between 50 to 60%, this figure in the Japanese population is only 11.5% and thus strikingly lower.

TABLE 1
Different gene frequency of INH-acetyl transferase

Population	no.	Acetylation of INH		Gene frequency Ac^S
		Ac^RR + Ac^RS	Ac^SS	
Europe	900	459 (51%)	441 (49%)	0,70
Africa	197	91 (46%)	106 (54%)	0,74
India	442	180 (41%)	262 (59%)	0,77
				0,76
				0,54
Japan	1808	1601 (88,5%)	207 (11,5%)	0,34
Thailand	100	43 (43%)	57 (57%)	0,76
Rhesus monkeys	106	60 (56%)	46 (44%)	0,69

Goedde, H.W. (4).

Using a high INH-dosage of more than 10 mg/kg in "slow acetylators" polyneuritic disturbances are observed eight times more frequently than in the group of "rapid acetylators" (4). This type of polyneuritis is probably caused by increased vitamin B₆-excretion. Simultaneous application of vitamin B₆ will prevent such a polyneuritis in most instances. Perhaps there exists a similar polymorphism for yet another group of substances such as sulfamidine (17), phenelzine (9), hydralazine (3) as well as serotonin (18) which are acetylated likewise by N-acetyltransferase. In leaving this point it should however be noted that the success of INH therapy is not dependent upon the velocity of acetylation.

2. *Pseudocholinesterase-deficiency*

A frequently used muscle relaxance in anesthesia is the drug succinylcholin. Application of 1 mg/kg succinylcholin results in general in an apnoea of 7 to 10 minutes. Systematic analyses have revealed that among 1500 patients only in one instance a dangerously extended prolongation of such apnoea for up to several hours can occur (5, 10, 20). As causative connection for such a prolonged apnoea the genetic variants of the serum enzyme pseudocholinesterase could be demonstrated. Pseudocholinesterases catalyze the two-step hydrolytic cleavage of succinylcholin via succinylmonocholin into succinate and cholin. In its chemical nature the enzyme is an acid glycoproteide of the alpha-2-beta serum globulines with a molecular weight of 365.000 dalton.

Extensive analyses of the pseudocholinesterase-activity in the serum of patients with prolonged apnoea after application of succinylcholin as well as of their family members resulted in the demonstration and identification of genetically determined variants of this enzyme in most instances.

Measurement of enzyme activity only by the routine substrate benzoylcholin does not allow the detection of the different variants. These activities are distributed in way of a unimodal fashion (Figure 2). Employing electrophoretic as well as chromatographic methods combined with the determination of affinity of substrates and the inhibition constants for dibucaine and fluoride (8, 11, 12) several phenotypes of the pseudocholinesterase have been successfully characterized. Besides the dibucaine and fluoride resistant enzyme variants in case of complete absence of enzyme activity the presence of a "silent gene" has been mentioned (1, 6, 15). Figure 3 demonstrates that the dibucaine resistant variant of pseudocholinesterase is not able to hydrolyse succinylcholin within the therapeutical range (10). Following Goedde (4) today a four allele model of the cholinesterase can be trusted. Its combination of alleles, characteristics, clinical significance and meaning are summarized in table 2. According to this knowledge four homozygous phenotypes exist as well as six different heterozygous

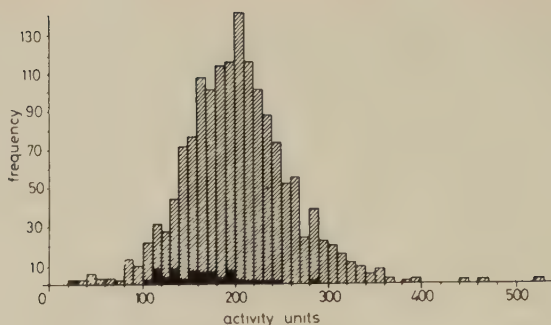


FIG. 2. Distribution of pseudocholinesterase activity units from 2032 persons. Black bars = heterozygotes. Kalow, W. and Staron, N. (12).

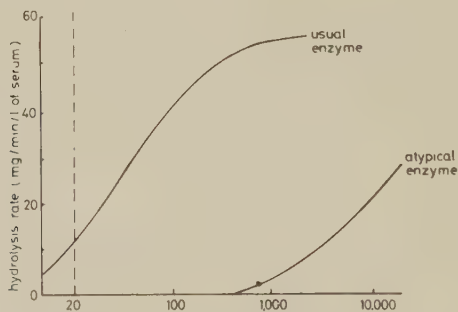


FIG. 3. Concentration of succinylcholine chloride in mg/l (log scale). The different rates of hydrolysis of suxamethonium by the pseudocholinesterase variants. The dotted line indicates the concentration found in vivo. Kalow, W. (1960), Cholinesterase types, in *Ciba Foundation Symp. on Biochemistry of human genetics*, G.E.W. Wolstenholme, and C.M.O'Connor, eds. London, J. and A. Churchill, Ltd.

carriers. The most common atypical variant A was associated with the most dangerous apnoea besides the S and AS phenotypes. This type A structure perhaps is characterized by an exchange of a dibasic acid for a neutral amino acid within the active center of the enzyme (4). In a persisting apnoea fresh plasma is indicated therapeutically. Considerably more effective however is a highly purified and lyophilized preparation of human pseudocholinesterase (5). It remains to be mentioned that also a 3 to 4 fold increase of pseudocholinesterase activity has been described (6). The carriers of variants showed a complete resistance for the therapeutic dose of succinylcholin.

TABLE 2
Model of Cholinesterase Alleles

Genotype		Pheno- type	Activ- ity	% inhibi- tion by dibucain	Clinical symptoms	Frequency
Eu ₁	Eu ₁	U	95	80	No	~96%
Ea ₁	Ea ₁	A	38	22	Strong prolonged apnoea after	~0.05%
Es ₁	Es ₁	S	0	0	Succinylcholin	~0.001%
Er ₁	Ef ₁	F	—	66	Prolonged apnoea	Very rare
Eu ₁	Ea ₁	UA	57	62		~3.6%
Eu ₁	Ef ₁	UF	62	74	No	Very rare
Eu ₁	Es ₁	US	50	80		Rare
Ea ₁	Es ₁	AS	20	22	Strong prolonged apnoea	Very rare
Ea ₁	Ef ₁	AF	—	49	Prolonged apnoea	Very rare
Ef ₁	Es ₁	FS	61	67		

Goedde, H.W. (4).

3. Disturbances of diphenylhydantoin-hydroxylation

In epileptics the side effects of diphenylhydantoin therapy in ways of ataxia, nystagm and lethargy can be due to overdose of that drug, but also due to pharmacon interaction in case of simultaneous application of isoniacid, chloramphenicol, dicumarole, disulfiram, phenyramidol, pheneturides as well as sulthiamme. The above drugs can reduce the hydroxylation of diphenylhydantoin as is examplified in figure 4 (17). Within this topic these causative mechanisms of increased concentrations of blood diphenylhydantoin shall not be discussed. Important in this context is only that the described neurological side effects may also arise with normal doses of diphenylhydantoin at 4 mg/kg body weight. Kutt *et al.* (14) have described one patient in whom during a daily dose of 3.8 mg diphenylhydantoin per kg body weight for two weeks the plasma concentration of this drug reached 70 µg/ml. Using the same dose in healthy individuals its concentration ranged from 4-8 µg/ml. The mother as well as the brother of the above patient had similarly augmented plasma concentrations of this drug.

In the body diphenylhydantoin is hydroxylated in paraposition by a microsomal hydroxylase of the liver, thus inactivated and consecutively 60-70% is excreted in the urine as p-hydroxy-diphenylhydantoin. Among the defect carriers the excretion rates of the hydroxylation product are clearly lower. Furthermore the accumulation of diphenylhydantoin in the plasma, liquor and saliva also is a consequence of the deficiency of microsomal liver hydroxylase. In figure 5 the increase of diphenylhydantoin concentration in a healthy control is compared

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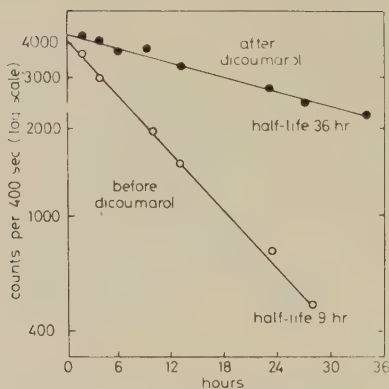


FIG. 4. Effect of dicoumarol and half-life of diphenylhydantoin in serum. Mølholm Hansen, J. *et al.* (17).

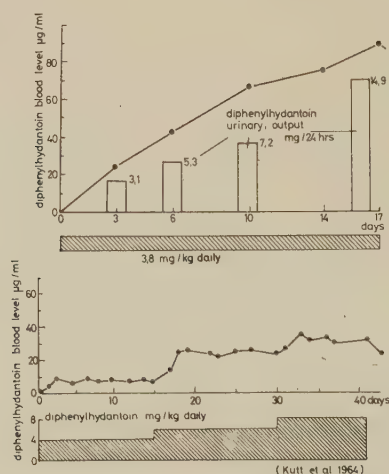


FIG. 5. Diphenylhydantoin levels in blood of patient W.J. while taking 300 mg/day. The bars represent the urinary output of unmetabolized drug in mg per twenty-four hours. Diphenylhydantoin levels in blood of average normal individual receiving various amounts of drug. Kutt *et al.* (14).

to that of a patient suffering from hydroxylase deficiency (14). This enzyme deficiency is probably inherited autosomally or dominantly linked to the X-chromosome. The same authors could not detect a disturbance of the p-hydroxylation of phenobarbital or of phenylalanine. Likewise no toxic effects could be found in the defect carriers under therapeutic dosis of phenobarbital.

4. Intolerance for acetophenetidin

Minor hemolyses and methemoglobinemias as well as interstitial nephritis can be observed occasionally in long term acetophenetidin abuse. It has been reported on a pair of siblings suffering from severe hemolysis as well as a methemoglobinemia affecting about 50% of hemoglobin combined with Heinz bodies after a calculated consumption of 2.5 g acetophenetidin (22) (Figure 6). The acetophenetidin metabolites mentioned create substantial amounts of methemoglobin in vitro. Occurrence of Heinz bodies, hemolysis and methemoglobin levels even increased, if phenobarbital had been given first for induction of microsomal hydroxylating enzymes. Concomitantly severe neurological symptoms appeared which subsided only 9 hours later. The author hypothesises that a genetic defect in the deethylation leads to an increased hydroxylation of acetophenetidin which is then augmented even further via an induction of microsomal liver hydroxylases.

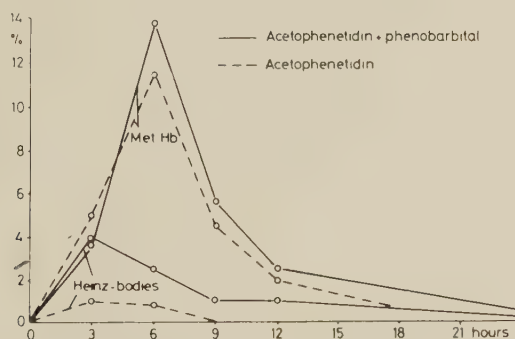


FIG. 6. Methemoglobin concentration and Heinz body formation after application of acetophenetidin (30 mg/kg). Shahidi (22).

In summary the examples shown above demonstrate that in the face of toxic side effects of drugs not simply overdose but also a disturbance of drug catabolism mediated by genetic enzyme deficiency has to be considered. Administration of more than one drug simultaneously can lead to a considerable potentiation of the intoxication via interactions between the drugs.

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Thyroid disorders due to various enzyme deficiencies

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INTRODUCTION

The synthesis of thyroid hormones depends on a series of specific enzyme systems. Any form of thyroid insufficiency not consecutive to anatomic dysgenesis or to athyreosis may be due to an alteration of one of these systems. Enzyme deficiencies are divided into *quantitative* disturbances present in various degrees in most acquired thyroid diseases and *qualitative* abnormalities detected in patients with congenital thyroid diseases and subdivided according to sequential thyroid hormone biosynthesis.

The first step of thyroid hormone synthesis is the active iodide uptake by the gland, which is energy-dependent, and requires activated ATPase. The iodide transported into the thyroid is rapidly oxidized, probably into a free radical (I^+), by peroxidase and H_2O_2 . Thyroid peroxidase (TPO) is a heme protein similar to Protoporphyrin IX which is present in the particulate fraction of the thyroid in a tetramer form. It can be reversibly degraded to a monomeric form that remains enzymatically active. The H_2O_2 source probably originates in the microsomal enzymes which involve reduced triphosphopyridine nucleotide (NADPH) and cytochrome C reductase (1). The same enzymes oxidize the tyrosine residue of thyroglobulin for the formation of moniodotyrosine (MIT) and diiodotyrosine (DIT) and also catalyze the next step: the coupling reaction between iodotyrosyl residues in thyroglobulin to form intra-thyroglobulin thyroxine (T_4), 3,5,3' triiodothyronine (T_3) and 3,3'5' reverse T_3 (rT_3).

The thyreostimulating hormone (TSH) stimulates all these steps, while anti-thyroid drugs inhibit iodide peroxidation.

The mature iodinated thyroglobulin formed is poured into the colloid. Under TSH stimulation, pinocytosis of colloid occurs and the colloid droplets formed are hydrolyzed by the catheptase liberated from their fusion with lysosomes. Proteolysis of (Tg) thyroglobulin results in the liberation of T_4 , T_3 , rT_3 , MIT and DIT. Under normal conditions, only T_4 , T_3 and small amounts of iodide are released into the circulation. MIT and DIT are rapidly deiodinated by a thyroid enzyme, deshalogenase (deiodinase). The iodide so generated returns

to the second thyroid iodide pool and represents intrathyroidal reutilization of I. The enzyme is specific for the halogen derivatives of tyrosine and does not deiodinate iodothyronine. Deiodination of tyrosine also occurs in other peripheral tissues (liver, kidneys, salivary glands).

The thyroid hormones liberated into the bloodstream are bound in a large proportion to serum proteins [thyroine-binding globulin (TBG), prealbumin, and albumin]. However, only the small unbound, free fraction ($< 0.1\%$), which is biologically active, penetrates into the cells and exerts its metabolic effects.

ALTERATIONS OF THYROID HORMONES BIOSYNTHESIS

Alterations in the formation of thyroid hormones caused by abnormal function of the enzyme systems involved have been detected in almost all thyroid diseases. *Quantitative* disturbances are present in goiters, cold and toxic adenomas, thyroiditis and carcinoma, and *qualitative* abnormalities are detected in patients with congenital thyroid diseases.

These patients usually present syndromes of hypothyroidism and goiter developing in early infancy, leading to growth retardation and mental subnormality if diagnosis and therapy are not promptly carried out. Lesser degrees of malformation are often associated with the appearance of goiter in adolescence and an euthyroid metabolic state. The patients tend to have larger vascular thyroids and accelerated radioactive iodide uptake and turnover. Clinical and laboratory studies have subdivided the affected subjects into categories coinciding with the sequential steps of hormone biosynthesis.

1. *Iodide trapping defect.* Until now only about 14 cases have been described (2, 3). The defect is characterized by the presence of goiter, a low thyroidal ^{131}I uptake, no concentration of iodide by the salivary glands. There is a specific defect in the iodide transport molecule, but the passive transport ability may be preserved. Replacement therapy with about a hundred times the normal daily intake of iodine (10-100 mg/day) restores hormone synthesis to normal, since the other mechanisms (TPO, thyroglobulin, deiodinase activity) are present and normal.

2. *Iodide organification defect.* The cause of iodination deficiency could be due either to the absence of H_2O_2 generation, of the prosthetic group, of peroxidase, to the presence of inhibitors or to abnormal iodoprotein substrate (1, 4, 5).

Iodination takes place at the apical border of the follicle cells. TSH controls the level of TPO enzyme, inducing specific enzyme formation and stimulating the formation of H_2O_2 . Indeed, peroxidase activity disappears 48 h after hypophysectomy. Migration of the enzyme to the apical border and its concentration

in apical vesicles strongly suggests that a "meeting" is going to take place between TPO, preformed Tg and iodide at the cell colloid interface.

Some antithyroid drugs inhibit the iodination of tyrosyl residues and excess of iodide inhibits iodine formation (Wolf-Chaikoff effect), but this inhibitory effect is transient because the iodide transport system in the thyroid adapts in some manner and limits intracellular iodide transport.

Thus one of the major physiological controls on hormone formation is the iodide supply, but, according to Taurog *et al.* (6), free DIT seems to play a role as well. Iodide deficiency, if any, will increase the ratios MIT/DIT and T_3/T_4 , but the total T_4 and T_3 formation will decrease, resulting in TSH stimulation and goiter.

The defect is characterized by:

- an early high thyroidal ^{131}I uptake. However, as this trapped iodide is neither oxidized nor bound, the administration of 1 g of potassium perchlorate or thiocyanate will discharge it;

- by a normal or reduced Tg content, but practically uniodinated.

The defect may be complete and lead to severe hypothyroidism or it could be partial. In this case, the enlargement of the thyroid is sufficient to compensate the defect and the patients are more or less euthyroid (5). It is also observed in Pendred's syndrome, where familial goiter is associated with nerve deafness.

3. Iodotyrosyl coupling defect. After iodotyrosine is generated in Tg, a secondary coupling reaction occurs leading to the formation of intrathyroglobulin thyroxine and T_3 and involving the coupling of the iodotyrosyl molecules. The mechanism suggested for the reaction is an oxidation leading to the formation of iodotyrosyl free radical or positively charged ion which can be transferred within thyroglobulin to another iodotyrosyl group to form iodothyronine (1, 6). The intramolecular rearrangement might be facilitated by maturation of the molecule and the formation of its tertiary and quaternary structure through the formation of disulfide bridges and ionic or hydrogen bonds (1).

The two major agents affecting the coupling reaction are iodide intake and TSH stimulation.

About 15 patients have been reported to date with presumed deficiency in the coupling process (1, 3). They have enlarged hyperplastic thyroid, elevated thyroidal radioactive iodide uptake with rapid iodide turnover and may have normal or depressed levels of thyroid hormones in blood. The presence of MIT and DIT and no T_3 nor T_4 on chromatographic analysis of *in vivo* labelled thyroid iodoproteins is the proof for the diagnosis. There was no evidence of consanguinity and it was found in males and females. In reality, the diagnosis is difficult, Tg being generally normal, but hypoiodinated despite the normal or high

iodide uptake. The patients with "coupling defect" should be classified into other subcategories. Indeed, alterations in the distribution of iodide among thyroid hormones and its precursors in the thyroid with reduction of the iodothyronine/iodotyrosine ratio are induced by a deficiency of peroxidase and also occur in iodide-deficient endemic goiter, in sporadic multinodular goiter and in thyroiditis. Thus, a deficiency in some aspects of the iodide peroxidase function is the cause of the coupling abnormality in most instances, but complete proof is lacking.

4. *Defects in the formation of thyroglobulin (Tg) and release of iodoproteins.* Thyroglobulin (Tg), a glycoprotein of molecular weight 660,000, is thus the substrate for the synthesis of thyroid hormones. It is initially composed of subunits 6S, 12S and 17S. Iodination of the 17S molecule leads to formation of 19S mature thyroglobulin with a change in Tg conformation. As iodine content increases, the DIT/MIT and T_4/T_3 ratios all increase. Coupling is apparently restricted severely at iodination levels below $250\mu\text{g I/g}$ thyroid or 0.1% I in thyroglobulin. However, in the presence of high Tg iodination, only few T_4 and T_3 residues are formed, indicating that only few iodotyrosyl groups are in appropriate position for coupling.

In addition to Tg, the thyroid contains some soluble iodoproteins: thyroalbumin, prealbumin or proteins related to Tg (12S, 27S). Normally, Tg is degraded before entering the bloodstream, but small amounts are detected in the circulation and represent the butanol insoluble iodine (BII) or nonbutanol extractable iodine (NBEI).

In the absence of normal Tg synthesis, the other proteins may be formed preferentially and iodinated in excess in the gland (7, 8, 9, 10). Usually, T_4 and T_3 contents of such abnormal proteins are much below those found in Tg. T_3 can be formed in a higher proportion than T_4 and be responsible for the euthyroid state. The turnover of such proteins is usually rapid. In one sibling, Savoie *et al.* (11) found an iodoalbumin in the gland which contained much mono- and diiodohistidine.

Kusakabe (8) has reported two apparently unique defects in Tg structure associated with organification defect. One of them showed evidence of Tg subunits and good formation of MIT, but defective ability to form DIT and thyroxine. The absence of Tg (9) and of defective Tg export (12) have been described.

Patients with a wide variety of thyroid abnormalities (Graves' disease, Hashimoto's thyroiditis, carcinoma of the thyroid) in addition to large congenital goiter have been reported with an increased proportion of BII in the blood, which was identified by different techniques both in thyroid and in plasma as 4S albumin, albumin-like prealbumin, subunits of Tg or heavy iodoproteins.

In addition, there is also, in certain cases, a proportional increase in particulate iodoproteins.

5. *Iodotyrosine desiodase defect.* Lack of desiodinase results in loss of iodotyrosine in blood and urine. In 1953, 12 cases were described in Scotland (13), then in 1955, Stanbury *et al.* (14) identified the defect. The patients usually present elevation of the early thyroidal ^{131}I uptake followed by rapid discharge of the isotope from the gland. A great proportion of serum radioactivity appears as MIT and DIT, with a smaller proportion of T_4 , T_3 and iodide. When ^{131}I -labelled DIT is given intravenously, virtually all the radioactive material is recovered unaltered in urine. There is thus a complete or partial defect of peripheral deiodination of DIT as well (15, 16).

All patients have thyroid enlargement. PBI could be within the normal range. The frequency with which malignancy or degeneration changes occur is high. It is an autosomal recessive type of inheritance without sex predilection. When pheno-typically normal parents or relatives had been tested for peripheral deiodination activity, a partial abnormality was demonstrated in some subjects. The loss of iodotyrosine results in a state of iodine deficiency, thyroid stimulation and hyperplasia. The correction of hypothyroidism can be performed by administration of iodine alone.

It is not known whether the defect is due to the absence of the enzyme desiodase or to its inactivation by the presence of an abnormal substance.

6. *Peripheral alteration of thyroid hormone action.* Almost every known aspect of peripheral cell metabolism is affected by thyroid hormones. They have stimulatory effects on many intracellular enzymes, on the metabolism of glucose, lipids, proteins and other hormones, on electron transport, synthesis of nuclei and cytoplasmic ribonucleic acid (17).

After penetration into the cell, thyroid hormones are bound to specific receptors in the nucleus at the chromatine, this binding being associated with their action (17, 18). It is thus possible to conceive that a decrease in the number of nuclear receptors or their absence will reduce thyroid hormone action.

Refetoff *et al.* (19) have described three children from consanguinity marriage who present deaf mutism, delayed bone age with stippled epiphyses, goiter and elevated circulating thyroid hormone levels in the presence of normal thyroxin binding globulin capacity and apparently euthyroid clinical status. The data obtained strongly suggest the presence of a congenital defect associated with variable degrees of tissue resistance to the action of thyroid hormones. This has been partially compensated by excess hormone production.

Finally, it is well known that, at equimolecular concentrations, T_3 is three to five times more active than T_4 . This higher T_3 activity is partially due to the

higher affinity for T_3 than for T_4 to nuclear receptors, leading to a higher T_3 nuclear concentration than with T_4 (20, 21). Moreover, a large proportion of the circulating T_3 is raised from peripheral deiodination of T_4 into T_3 by iodothyronine desiodase. However, there are probably two distinct desiodases, one leading to T_3 (5' desiodase), the other to rT_3 (5 desiodase) (22, 23), which is an isomer of T_3 biologically inactive. T_3 seems to inhibit 5 desiodase; but, under certain conditions (acute and chronic diseases, during fasting and under certain drugs...), there is a predominant activation of 5 desiodase leading to reduced formation of T_3 and to an increased one of rT_3 . This represents a form of slight peripheral hypothyroidism (24, 25, 26).

In conclusion, the etiology of congenital defects in thyroid hormone synthesis being now well documented, allows us not only to know the enzymatic systems involved in normal thyroid hormone synthesis, but also to understand the disturbances responsible for the various acquired thyroid diseases in which the same processes are altered, however to a lesser degree.

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SYMPOSIA
CONTROVERSIES IN GASTROENTEROLOGY

Lack of fiber as a cause of intestinal diseases

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In brief, I cannot fully explain the hypothesis linking lack of fiber and intestinal disease, let alone give all the evidence and arguments. All I can do is summarise the major arguments.

The best-known theory, recently popularised and extended by Denis Burkitt, states that small solid colonic contents are pathogenic to the colon for several reasons. *Firstly*, they are difficult to *propel*. Therefore, they force the bowel to generate excessively high pressures. Colonic hypertension is especially severe if segmentation around a small stool results in closed chambers.

Neil Painter has shown by combined cinematography and pressure measurement that in such closed chambers very high pressures can develop, and he has postulated that diverticula are simply blow-outs at the weakest points in the wall.

He has suggested that both diverticular disease, at least of the classical sigmoid type, and colon spasm or irritable bowel syndrome result from the colon being "irritated", or forced to struggle with unphysiologically small stools.

Similarly Burkitt has postulated that appendicitis is due to a faecolith—or abnormally solid lump—or to spasm obstructing the neck of the appendix.

Secondly, small, solid stools are difficult to expel, forcing the subject to strain at stool.

Straining is performing the Valsalva manoeuvre. This raises intra-abdominal pressure to as much as 200 mm mercury.

According to Burkitt, this can cause haemorrhoids by obstructing flow in the rectal veins or by prolapsing the anal cushions.

Thirdly, small stools are concentrated stools. With concentrated stools there is more intense exposure of the colonic mucosa to carcinogenic or toxic substances within the stool. According to Burkitt and Walker, this is the main dietary mechanism for cancer of the large bowel.

Fourthly, small solid stools move slowly round the colon. This allows increased bacterial formation of carcinogens and toxins.

A central assumption in this theory is that small, solid stools are generally due to a low intake of dietary fiber.

A complementary theory incriminates the refined carbohydrates sugar and white flour, which are properly regarded as fiber-depleted foods. These, it is

said, promote inflammatory diseases and cancer of the intestine, probably by altering the bacterial flora. I have suggested that they promote cancer of the bowel by causing overnutrition or energy imbalance.

The grounds for these theories are 5-fold: historical, geographical, case-control diet comparisons, animal experiments and the effects of adding fiber to the diet.

Historical evidence is necessarily scanty but suggests that both diverticular disease and appendicitis were rare before the 20th century and became much commoner during the early years of the century. Crohn's disease was unrecognized till 1932. In Europe, it has increased several-fold since world war 2. During the 20th century the diet of the common people has changed more rapidly than at any time in history. The biggest change has been from starchy staple foods to sugary foods and drinks. This has meant a sharp fall in cereal fiber and a rise in refined sugar or sucrose consumption.

Geographical evidence is not easy to interpret, but it seems generally true that in any population where the habitual stools are bulky and soft, intestinal diseases other than infections are uncommon, for example rural Africa, rural India and rural Japan. Conversely, wherever stools are small and firm, intestinal diseases are common, as in most of Europe, North America including the blacks and second generation Japanese, and other affluent countries.

Appendicitis has been shown to be unexpectedly *uncommon* in Western countries under certain conditions, namely in children's homes or orphanages, in prisons and during wartime. In each situation, the diet is unusually coarse, with more porridge, brown bread and vegetables, and less cakes, biscuits and sweets.

Large bowel cancer is 4 times commoner in urban Denmark than in rural Finland, although the two populations are genetically similar. When the International Agency for Research on Cancer compared the diets of randomly selected men in the two populations, the main difference was in the intake of dietary fiber. This was 30.9 grams per day in Finland and 17.2 g/d in Denmark. Fat intakes were similar.

Experimentally, increasing the fiber content of the diet has several effects all of which are likely to be beneficial. Stool weight is increased, its consistency is softened, slow transit is accelerated and high pressures are lowered. All these effects suggest that the work of the large bowel is made easier.

Workers in Edinburgh have measured the intrasigmoid pressures in patients with diverticular disease in 3 situations, fasting, after food and after injection of neostigmine. In each situation, pressure was measured before and during treatment with bran, and in each situation pressure was substantially lower during bran.

If adding fiber to a low residue diet has these beneficial effects then it can be assumed that removing fiber from a high residue diet will have the opposite effects. It will reduce stool weight, slow down transit and increase pressures. All these effects increase the work of the large bowel and indicate it is inefficient.

In conclusion, the colon exists to receive and process undigested food residues. These residues, which are its *raison d'être*, consist chiefly of fiber. Man's colon, like that of all animals, evolved to function optimally on a coarse, unprocessed diet. In almost all cases this is a fiber-rich diet. Refined or fiber-depleted foods were invented too recently for evolutionary adaptation to have had time to occur.

I submit to you that it is only rational if not inevitable that such unnatural foods will be pathogenic to the large intestine.

Medical management of Crohn's disease

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There are two ways I can approach this presentation. One, is to show slides full of data from numerous publications on the subject, try to reconcile all of the conflicting results and opinions, and end with some consensus. The other, is to discuss some of the problems inherent in the evaluation of therapy in Crohn's disease, note strategies that might be employed to overcome these problems, and describe one attempt to do so. I have chosen the latter course.

Some of the factors in Crohn's disease that influence evaluation of treatment are shown on the first slide.

Crohn's disease factors influencing evaluation of therapy

Etiology unknown

Prolonged course

Spontaneous relapses and remissions

Protean manifestations

Physicians and patients discouraged

The etiology of Crohn's disease remains unknown. Interesting leads are being pursued in areas of immunology and infection, especially viral, but no breakthrough is on the horizon. As in most diseases, lacking a known cause we also lack a specific cure. Thus, we are thrust into the position of managing the disease as best we can.

The disease naturally runs a prolonged course, lasting many years. There are frequent spontaneous relapses and remissions. The manifestations are protean, many bodily functions outside the intestinal tract are altered, and some or all of the clinical aspects come and go in mystifying ways. In this setting of a chronic disabling disease of unknown cause and varying expressions, both physician and patient become discouraged, both seize upon any new treatment with great hope, and hope is sometimes a powerful therapeutic tool.

Crohn's disease modes of therapy available

Drugs: antimicrobial, anti-diarrhea, anti-inflammatory, immunosuppressive

Diets: general, restricted, elemental, intravenous

Rest

Surgery

The modes of therapy available today fall into 4 groups—drugs, diets, rest, and surgery. Four classifications of drugs are used—antimicrobials, anti-diarrhea, anti-inflammatory, and immunosuppressive. Highly restricted diets are not as widely used as before, except in selected circumstances. Good nutrition is paramount. Both elemental diets and intravenous alimentation are sometimes needed to achieve this. Rest in the acute phase of the illness has become axiomatic, along with good nutrition.

While it is true that some patients have no further trouble after extirpation of the diseased bowel, it is equally true that some patients have the same result following medical therapy or following no specific therapy. Furthermore, since surgery is not curative in any reasonably consistent manner and since operation is fraught with both immediate and long term hazard, the general tendency has been to attempt to tide patients over the acute episode with non-surgical treatment, reserving operation for those patients with complications or life-threatening continuance of the exacerbation.

If this be our philosophy what treatments do we use? This is a difficult question to answer because there are so many problems inherent in published reports, both of medical and surgical treatment. The vast majority of the reports

Crohn's disease problems in published reports of treatment

Anecdotal	Multiple drugs
Few patients	Single institution
Short followup	Imprecise criteria for status change

are anecdotal, do not describe enough patients, nor have the patients been followed long enough. A study drug is often given or a surgical procedure done in the presence of other drugs that are also alleged to improve the disease, making evaluation of the test treatment difficult or impossible. Most studies are reported from a single institution. While this has certain advantages, it also has important disadvantages. The patient population may be badly skewed—toward ethnic or religious background, economic status, or, in very homogenous populations, genetic makeup. The physician himself may be a powerful therapeutic tool and bias results, especially in poorly designed studies. Lastly, few studies have attempted to define precise criteria for change in disease activity or patient well-being using specific indicators and arriving at some standardized assessment.

With so many problems apparent, what characteristics should we look for in a treatment trial?

The study must be carefully designed, the patients randomized among treatment and control groups, and evaluated in a double-blind fashion. Large numbers of patients are required and the followup must be as long as possible—

Crohn's disease suggested characteristics of a treatment trial

Carefully designed study	Single drug treatment, or specific combined treatment
Randomized	Precise criteria for status change
Double-blind	Multi-center trial
Large number of patients	
Long followup	

the longer the better. A single treatment or a specific combination should be compared to the control. Precise criteria should be established for evaluating change. In a disease such as Crohn's these criteria almost mandate that a multi-center trial be used. Multi-center trials have their own problems, most of them revolving around the variations with which the clinical investigators at the various centers approach the diagnosis, interpret the protocol, and follow the protocol. Careful planning, centralized control of the data, and constant monitoring can minimize these potential faults. On balance, I believe that proper multi-center trials offer the best opportunity to evaluate treatment in a disease like Crohn's.

Such a trial has been completed in the United States and at least two others are ongoing in other countries.

Crohn's disease national cooperative Crohn's disease study

Prospective, randomized double blind	Placebo comparison
14 centers - 673 patients	1 to 2 year followup
8 disease parameters monitored (CDAI)	Pathology and x-rays read centrally
Single drugs used	Subgroups analyzed

The U.S. trial is known as the National Cooperative Crohn's Disease Study. Except for one paper describing the development of the Crohn's Disease Activity Index the results have been published only in abstract form. The trial is a prospective, randomized, double blind study with tight statistical control and with continuing scrutiny by a group of experts not involved in the study. Fourteen medical centers participated and 673 patients were studied. Eight disease parameters were measured in each patient at each visit to a study center. These were converted to a standard score—the Crohn's Disease Activity Index. Patients were treated with a single drug and results compared to a placebo treated group. The followup was 1 to 2 years. Pathology slides and x-rays were read centrally by a small group of pathologists and radiologists. Numerous subgroups were analyzed to detect treatment efficacy that might have been obscured in analysis of the total group.

Crohn's disease national cooperative Crohn's disease study

Part I, Phase 1: 295 patients, active disease prednisone, sulfasalazine, azathioprine, or placebo
17 weeks study period

Results: prednisone and sulfasalazine > placebo, azathioprine not > placebo,
placebo: 49% remission, 30% maintained

In Part I, Phase 1 of the study 295 patients with active disease were randomized to treatment with prednisone, sulfasalazine, azathioprine, or placebo. The study period was 17 weeks. Both prednisone and sulfasalazine were superior to placebo in inducing a remission but azathioprine was not. Of great interest in the interpretation of any therapy of this disease is the fact that 49% of patients taking only placebo went into a remission and 30% maintained the remission throughout the 17 weeks of this phase of the study.

Crohn's disease national cooperative Crohn's disease study

Part I, Phase 2: 95 patients, responded Phase 1 continued on effective drug 1-2 year study
period

Result: no drug > placebo

In Phase 2 of Part I, 95 patients who responded to Phase 1 therapy were maintained on that same drug but at a reduced dose for 1 to 2 years. None of the drugs were superior to placebo in maintaining a remission.

Crohn's disease national cooperative Crohn's disease study

Part II: 284 patients, prophylactic drug use disease quiescent, or patient post-op without disease
1-2 year study period

Result: no drug > placebo

Part II was designed to assess the effect of drug therapy in preventing relapse. 284 patients who had quiescent disease or had recently undergone extirpative surgery were randomly assigned to one of the four treatment groups and followed 1 to 2 years. No drug was superior to placebo in prophylactically preventing relapse.

Crohn's disease national cooperative Crohn's disease study

Part III: 89 patients, active disease, comparing prednisone + placebo to prednisone +
sulfasalazine

Result: no difference

Following the determination that prednisone and sulfasalazine were useful in bringing about a remission in active disease, Part III was designed to evaluate the relative effectiveness of prednisone plus sulfasalazine and prednisone plus placebo in inducing a remission. Prednisone plus sulfasalazine was not superior to prednisone plus placebo, thus no additive effect was demonstrated.

Crohn's disease national cooperative Crohn's disease study

General summary

Inducing remission: prednisone or sulfasalazine > placebo, prednisone + sulfasalazine
prednisone + placebo, azathioprine not > placebo
Maintaining remission: no drug > placebo
Preventing relapse: no drug > placebo

The main results of the trial can be summarized as follows:

Inducing a remission:

prednisone and sulfasalazine are more effective than placebo
prednisone plus sulfasalazine not more effective than
prednisone plus placebo
azathioprine not more effective than placebo

Maintaining a remission:

no drug superior to placebo

Preventing relapse:

no drug superior to placebo

Crohn's disease national cooperative Crohn's disease study

Other results

- 1) active disease: ileum alone - only prednisone > placebo, colon alone - only sulfasalazine > placebo, ileum and colon - prednisone or sulfasalazine > placebo
- 2) immunocompetent patients reacted same as anergic patients
- 3) only prednisone associated improved x-rays
- 4) disease complications - drugs not > placebo

Many additional findings will be reported from this study. I will mention only a few. Analysis of patients with active disease treated in Part I, Phase I reveals that those with disease localized to the ileum responded only to prednisone and not to the other study drugs, those with disease localized to the colon responded only to sulfasalazine, and those with combined small bowel and colon disease responded to both prednisone and sulfasalazine.

Those patients with positive skin tests used to screen for immunocompetence reacted to drug treatment the same as those with anergic response to skin tests.

Prednisone was the only drug associated with an improvement in intestinal x-rays.

No drug was superior to placebo in treating complications of the disease, including fistulae and peri-anal abnormalities.

Crohn's disease national cooperative Crohn's disease study

Adverse drug effects

- 1) patients off study: placebo 1%, prednisone 5%, sulfasalazine 6%, azathioprine 9%,
4 ↓ WBC, 3 pancreatitis

Adverse effects were found with all of the drugs, including placebo. More patients had to be removed from the study because of side effects from azathioprine than any other drug. As might be expected some patients developed leucopenia, but it was somewhat surprising that 3 developed pancreatitis, a complication of azathioprine that has been reported by others.

Crohn's disease - Suggested treatment

Active disease

- | | |
|---|--|
| 1) rest | 4) anti-diarrheal - codeine, Lomotil |
| 2) nutrition - food, elemental, intravenous | 5) prednisone 0.5-0.75 mg/kg, or sulfasalazine 1.0 g/15 kg |
| 3) vitamins, iron, transfusion | 6) cholestyramine, if indicated |

On the basis of this and other well-designed studies we can recommend the following medical treatment program. In patients ill with active disease the cornerstones of rest, adequate nutrition, blood replacement, and anti-diarrheal agents, including cholestyramine when indicated, should be our first thoughts. In patients ill with disease in the small bowel, or in both the large and small bowel, prednisone is probably the drug of choice. In those with only colitis, sulfasalazine may be more effective.

Crohn's disease - Suggested treatment

Quiescent disease or after extirpative surgery

- | | |
|-----------------------------------|------------------------------|
| 1) activity as normal as possible | 4) cholestyramine, if needed |
| 2) nutrition | 5) no specific drug |
| 3) anti-diarrheal, if needed | |

Patients who have quiescent disease or have recently recovered from extirpative surgery should be treated symptomatically with careful attention to nutrition

and should be encouraged to maintain as close to normal activity as possible. There is not now available any drug that is of specific benefit to these patients and since all of the drugs currently used to treat Crohn's have significant severe side-effects there is added reason not to use them. We must keep in mind the natural history of this disease, as is well illustrated by the large number of patients who had a favorable response coincident with placebo administration. Curative therapy is not in sight.

Early operation in Crohn's disease, yes or no

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The high incidence of recurrence in most reports over the years has convinced many physicians and surgeons to avoid surgical interventions as far as possible and that the place for surgery in Crohn's disease is mainly for patients with severe symptoms or for managements of complications. In many places therefore the general trend is towards medical treatment in the first instance and in recurrent disease this conservatism is increased. On the other hand, although experience has shown some benefit of steroids and salazopurin in the treatment of the disease, causing initial remission, medical treatment has mostly been a failure in the long run and the vast majority of patients will come to surgery eventually. Treatment with azathiaprine, metronidazole or recent trials with hyperalimentation has also been disappointing and has not changed this trend.

The main arguments against surgery generally stated are the high recurrence rate, the associated morbidity and mortality and the fear of postoperative malabsorption—after repeated resections eventually leading to short-bowel syndrome. Creation of an external intestinal stoma is another frightening consequence.

When expressed in cumulative figures, calculated by means of actuarial methods, the published results on recurrence rates after resectional surgery are in fact very consistent. It appears that during the first five years the yearly risk of reoperation for recurrence after the primary operation is about 10 per cent for small intestinal and ileocolic disease and 4 per cent for Crohn's colitis (Fig. 1). It is often stated that the risk for further surgery is increasing after each operation, but so far this suggestion is unproven and there is no evidence that surgery for recurrent disease is frequently the start of continuing complications.

As regards surgical mortality most reports show figures of similar magnitude (Fig. 2). To some extent, these figures, which are rather high, may reflect the nature and extent of the surgery involved. Moreover, the postoperative morbidity, which is also disappointingly high in many reports, suggests that many patients come late to surgery and are often in a reduced state of health with a variety of complications already occurred. Although there is no conclusive evidence it appears that both the postoperative mortality and morbidity might be reduced if the patients are brought to surgery earlier.

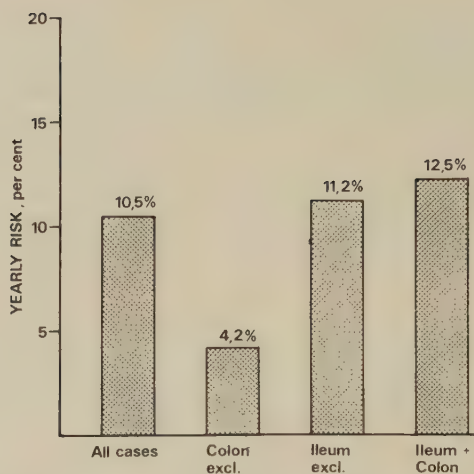


FIG. 1. The annual risk of recurrence in Crohn's disease as related to the site of the primary lesion.

<i>Author</i>	<i>Year</i>	<i>No. of major operations</i>	<i>Patient mortality</i>
			%
Van Pater <i>et al.</i>	1954	564	5.5
Colcock <i>et al.</i>	1960	346	2.0
Barber <i>et al.</i>	1962	325	3.1
Gjone <i>et al.</i>	1966	57	5.5
Lennard-Jones <i>et al.</i>	1967	91	6.8
Banks <i>et al.</i>	1969	213	7.5
Edwards	1969	244	6.2
Cooke	1971	459	7.1
De Dombal	1971	415	9.9

FIG. 2. Reported postoperative mortality in Crohn's disease.

Thus, in the Göteborg series where the general trend has been towards earlier surgery the mortality is only 1-2% and when comparing the overall complication rate after surgery in patients with and without preexisting complications to the disease a significantly lower morbidity in the latter group could be demonstrated (Fig. 3).

Aggravation of malabsorption has often been put forward as a heavy argument against surgery. However, although many patients may in this respect deteriorate markedly after surgery, some remain unchanged and many even

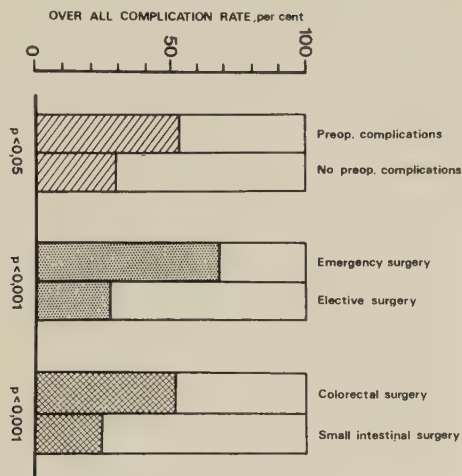


FIG. 3. Factors affecting postoperative morbidity.

improve (Fig. 4). Moreover, even in patients where surgery has resulted in malabsorption, as determined by conventional functional tests, there is still often a dramatic improvement in the patient's general condition and nutritional status. Admittedly, in patients who have already lost a considerable part of their small intestine from previous resections you might come into conflict when a further recurrence develops. Is there yet enough small bowel left for survival? Fortunately this situation is very uncommon and in our experience the number of patients with a "short bowel syndrome" developing eventually after multiple resections, has been small.

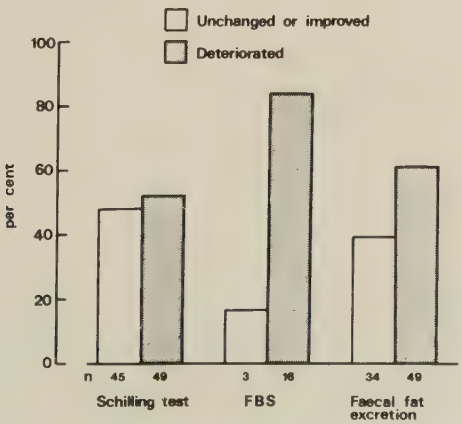


FIG. 4. Individual comparison of intestinal absorption before and after surgery for Crohn's disease.

Distal small bowel resection will give rise to a variety of functional disturbances, which must be taken into account when evaluating results after surgery. Along with increasing length of distal small intestinal resection the resulting absorptive dysfunction goes over a socially inconvenient choleroic, "watery" diarrhoea after short resections to a more profound disturbance of the entero-hepatic circulation with gross steatorrhea, fatty acid diarrhoea, hyperoxaluria, loss of fat soluble vitamins, calcium, magnesium and electrolytes, etc. Varying therapeutic measures have been suggested to lessen this sort of postoperative morbidity. Cholestyramin, Codein phosphate, MCT etc. have been tried, the result being sometimes beneficial but often disappointing. During the last years we have studied the effect of low fat diet (< 40 g/day) in these patients as the only or main measure and the result has been highly successful. The effect of fat reduced diet on the bowel habits showed a striking reduction in the number of diarrhoeas in post patients, particularly in these with the major part of the colon preserved (Fig. 5). Expressed in more objective terms both faecal water

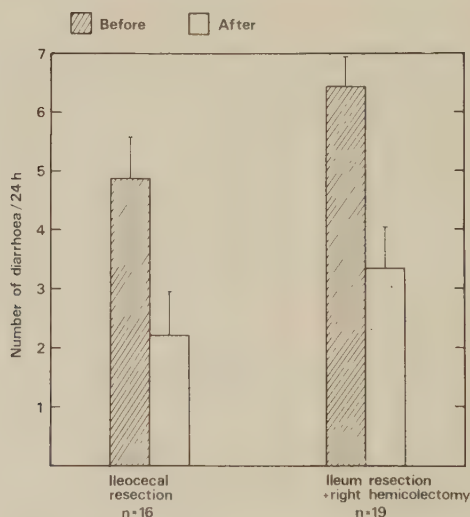


FIG. 5. The effect of fat reduced diet on bowel habits.

and bile acid excretion diminished markedly (Fig. 6 and 7). Moreover, the urinary oxalate concentrations was normalized in most patients (Fig. 8).

As regards recurrent disease, morbidity and postoperative morbidity the results of surgery for Crohn's disease are in many respects still unsatisfactory, but when compared with medical treatment, the results in terms of symptomatic relief and quality of life are not that bad after all and has in fact a lot to offer.

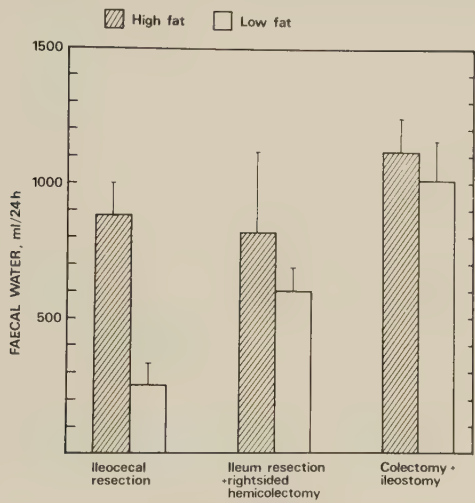


FIG. 6. The effect of fat reduced diet on faecal water content in patients with varying length of colon resection.

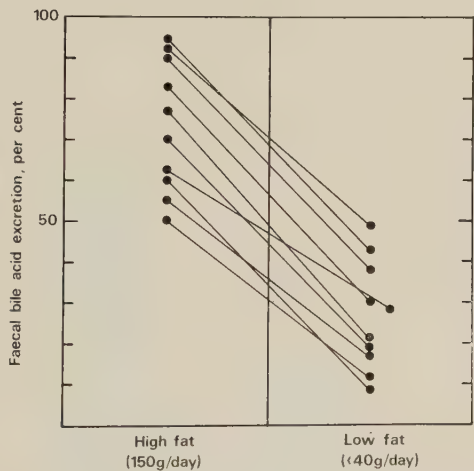


FIG. 7. The effect of fat reduced diet on faecal bile acid excretion.

Although there is a high recurrence rate after operation and reoperation, what is still encouraging is the greatly prolonged period spent in remission after each surgical intervention when compared to that offered by medical treatment.

Thus, it has been shown that about 70 per cent of all patient years are spent in full clinical remission of symptoms after surgery, compared to less than 20

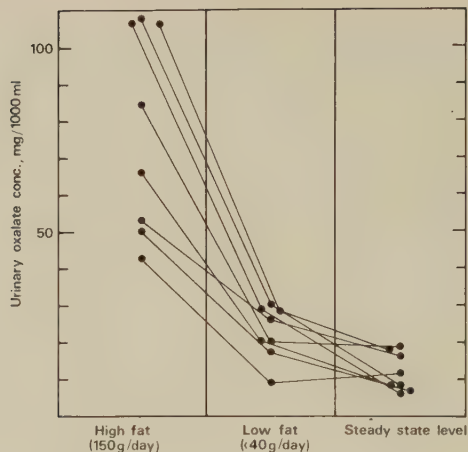


FIG. 8. The effect of fat reduced diet on urinary oxalate concentration.

per cent on conservative management (Fig. 9). In children with Crohn's disease a greatly prolonged relapse time after surgery as compared to medically induced remissions has also been reported (Fig. 10).

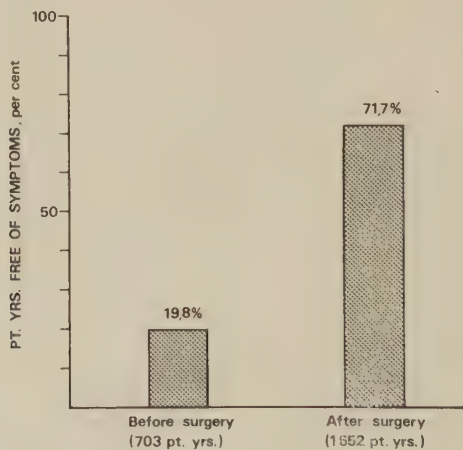


FIG. 9. Comparison of symptomatic course of Crohn's disease on conservative and after surgical intervention (de Dombal *et al.*, 1971).

The symptomatic relief and improvement in general health after operation, which is often most gratifying and may endure for many years, is reflected both in subjective and objective estimates. When the patient's own assessment of the results of operation is evaluated, it has been convincingly shown that the vast

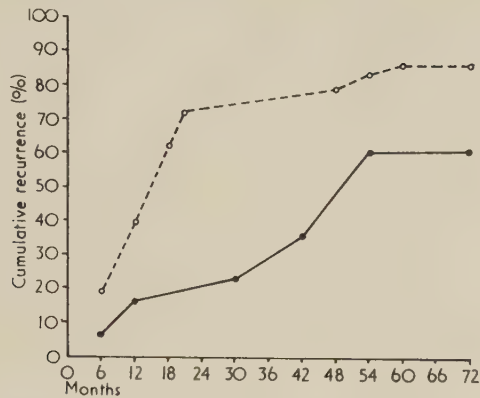


FIG. 10. Relapse rate after medical treatment. MRT 16 months (○); relapse rate after surgery, MRT 48 months (●) (O'Donoghue & Dawson, 1977).

majority of the patients are entirely satisfied, or satisfied with some minor reservations (Fig. 11). The effect of surgery based on objective estimates confirms these subjective statements and shows often a marked improvement in most metabolic variables with reestablishment of a good over all nutritional status (Fig. 12-16). Taking into account that malabsorption often increases in many patients after resectional surgery, these observations would imply that the activity of the disease itself is a more important cause of nutritional failure than is loss of small bowel.

If the major part of the colon can be preserved, and that is often the case in this disease, even wide or multiple distal small bowel resections are comparatively well tolerated or at least are the consequences more easily dealt with.

State of general health	Per cent of cases
Very good	46,3
Good	40,9
Fair	11,6
Poor	1,2

FIG. 11. General health at review in 243 patients after operation for Crohn's disease (Goligher *et al.*, 1971).

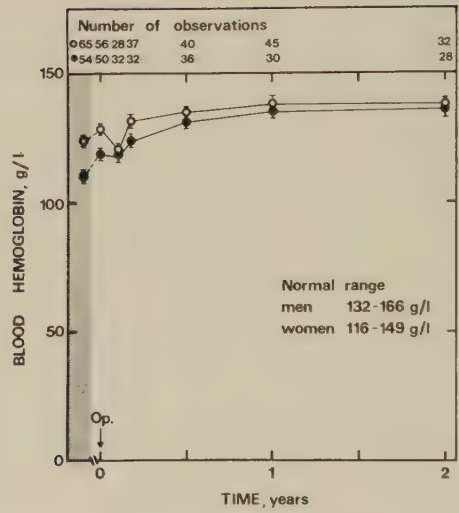


FIG. 12. Hemoglobin before and at intervals after surgery. ○ Ileal-predominantly ileal disease, ● colon-predominantly colon disease. (Shaded area, preoperative status).

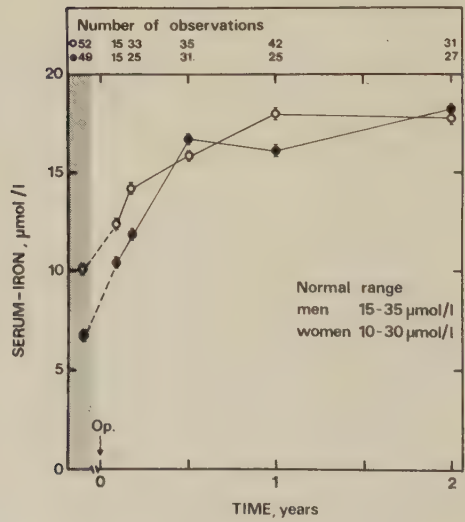


FIG. 13. Serum iron concentration before and at intervals after surgery.

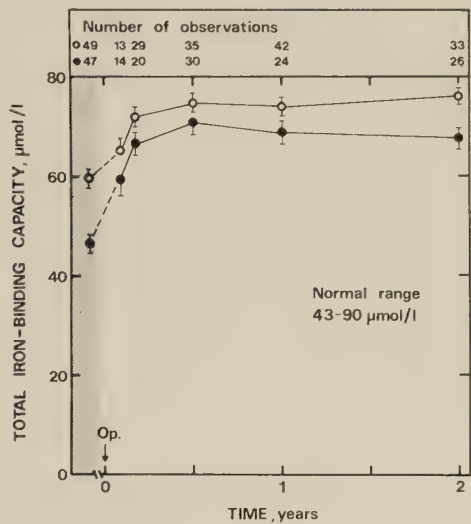


FIG. 14. Total iron-binding capacity before and at intervals after surgery.

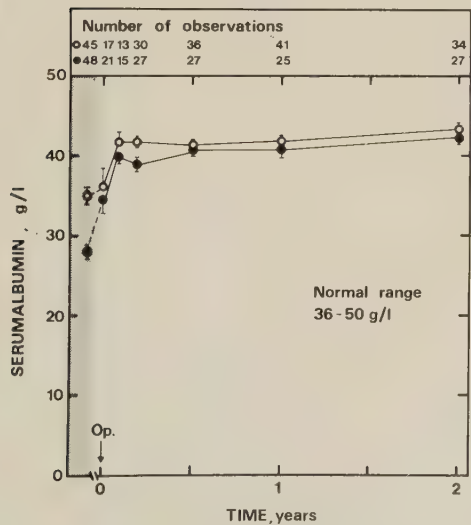


FIG. 15. Serum albumin before and at intervals after surgery.

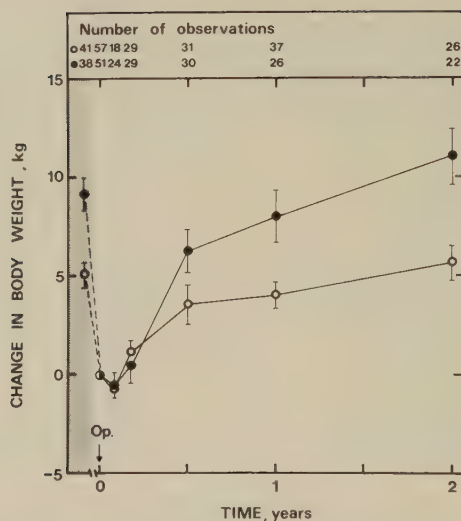


FIG. 16. Pre-illness weight and weight changes at intervals after surgery (body weight at operation set at zero level, shaded area, preillness weight).

CONCLUSION

The usually accepted indications for surgery in Crohn's disease is intractability to medical treatment (i.e. severe diarrhoea, weight loss and nutritional disturbances, physical and mental decline in the general condition) intestinal obstruction, intraabdominal abscess formation, external and internal fistulae, free perforation and severe perineal disease. The common statement that surgery should otherwise be avoided as far as possible must be an unfortunate philosophy.

Such a policy would allow many patients to continue to deteriorate to a stage when the intraabdominal state and the patient's general condition make surgery hazardous. What during an early phase of the disease would be a simple surgical problem might when surgery is delayed be a formidable undertaking. Undue delay often allows the extension of the disease to adjacent viscera. In this context it is important to stress that there is no evidence so far to indicate that increasing the duration of conservative treatment increases its efficacy. Moreover, the activity of the disease itself is a more important cause of nutritional failure than is loss of small bowel.

When conservative treatment fails an earlier resort to surgery, rather before complications have developed, will if anything improve the overall results after surgery, both as regards mortality and morbidity.

It must be realized that the illness often begins in early life, in one half during the twenties and in 90 per cent between 10-40 years. The increased time in terms of relief of symptoms obtained by surgery will get many children through the growth spurt of puberty and the formative years of education and even in young adults and adult patients, this has important implications, because the disease may have serious effects on social and family life or choice of career. There is so far no medical remedy of proven worth in the long term management of this disease. Although surgery does not cure the disease, operation and reoperation as required is often safer than the dangers of long-term medical treatment.

Corticosteroid treatment of HBsAg positive chronic active hepatitis

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INTRODUCTION

Our knowledge about benefits and risks of corticosteroids in chronic liver disease is mainly founded upon four controlled trials (1, 4) listed in Table 1. Since the presenting features of HBsAg positive chronic active hepatitis are clearly different from HBsAg negative chronic active hepatitis (4, 5, 6), it seems logical to re-examine the effect of corticosteroid therapy separately in the two subgroups of chronic active hepatitis. Unfortunately, data on the hepatitis B antigen status are only available from two studies (3, 4); and of these only the Mayo Clinic trial contained more than 10 HBsAg positive patients, with 12 patients in treatment programs including prednisone and 3 patients in the control group. It is evident that insufficient data are available at present to determine the benefit-risk ratio of prednisone treatment in HBsAg positive chronic active hepatitis.

TABLE 1

Prednisone for chronic liver disease. Incidence of HBsAg in controlled trials, 1977

<i>Place</i>	<i>No. of patients</i>	<i>HBsAg pos</i>
London (R.F.)	47	?
Rochester (M.C.)	136	13%
London (K.C.)	47	4%
Copenhagen	512	?

Results of prednisone treatment in HBsAg positive, and negative chronic active hepatitis

Results of treatment in patients with severe¹ chronic active liver disease (CALD) participating in the controlled Mayo Clinic trial are summarized in Table 2. In HBsAg negative CALD, clinical and biochemical remission occurred

¹ Severe: SGOT $> 10 \times$ upperlimit of normal, or SGOT $> 5 \times$ upper limit of normal in combination with gammaglobulin $> 2 \times$ upper limit of normal.

TABLE 2

Prednisone for severe chronic active liver disease - Mayo Clinic Study

	<i>Prednisone</i>		<i>Control</i>	
	<i>Clin/biochem. remission (71)</i>	<i>Treatment failure (18)</i>	<i>Clin/biochem. remission (9)</i>	<i>Treatment failure (15)</i>
HBsAg pos	6	6	1	2
neg	65	12	8	13

more frequently than treatment failure in prednisone treated patients; the opposite results were found in patients receiving control tablets. The beneficial effect of prednisone is highly significant in this group. In HBsAg positive patients, remission was found as frequently as treatment failure during prednisone treatment, while the number of patients in the control group is too small for assessment. These findings do not allow comparison of prednisone versus control therapy in HBsAg positive CALD, but do suggest a less beneficial response to corticosteroids in HBsAg positive CALD than in HBsAg negative CALD. In order to examine this detail, HBsAg positive patients were matched with HBsAg negative CALD patients to eliminate differences in presenting features such as sex, age, and immunoserology (ANF, ASM, etc); comparison of prednisone treatment in the two matched groups showed a significantly poorer response in the HBsAg positive CALD patients (4).

Recently results of long-term prednisone treatment of non-cirrhotic, moderate² chronic active hepatitis (CAH) have been published from Louvain, Belgium (7). Several years after initiation of prednisone treatment in patients admitted to the study with uniform entry criteria, HBs antigen testing became available. By chance two groups of equal size emerged allowing comparison of treatment response in HBsAg positive and HBsAg negative patients. Death and development of cirrhosis occurred more frequently in HBsAg positive CAH than in HBsAg negative disease. Using these endpoints of death or the development of cirrhosis, results of prednisone therapy in patients with severe CALD or moderate CAH are surprisingly alike (Table 3); the time span to reach these endpoints was however markedly different.

It seems therefore highly probable that prednisone treatment is less beneficial in HBsAg positive chronic active hepatitis than in HBsAg negative chronic

² Moderate: asymptomatic, with chronic aggressive hepatitis on liverbiopsy showing only slight piecemeal necrosis and no overt bridging necrosis.

TABLE 3

Prednisone for chronic active hepatitis - HBsAg pos vs HBsAg neg

	No.	Death	Cirrhosis developed
HBsAg pos: severe	13	4	2/6
		30%	61%
moderate	17	5	12/17
HBsAg neg: severe	13	1	3/10
		7%	31%
moderate	16	1	5/17

active hepatitis; preliminary results from a German multicentre trial provide additional evidence in this respect (8).

Possibility of a detrimental effect of prednisone in HBsAg positive chronic active hepatitis

Since immunosuppression might facilitate viral replication, prednisone treatment could be harmful to HBsAg positive patients (9, 10). Analysis of cumulative survival of the control group of severe CALD shows a 50% mortality at two years; HBsAg positive patients receiving prednisone fared better than control patients with a 30% mortality at 2 years. The absence of a HBsAg positive control group of adequate size makes a firm conclusion impossible, but the data suggest no harmful effect of prednisone.

Prognostic features with regard to response to prednisone therapy

Since about 50% of HBsAg positive patients responded to prednisone treatment with clinical and biochemical remission within a year, identification of prognostic features seems of great importance. Comparison of initial features of HBsAg positive severe CALD patients responding to treatment with those failing prednisone therapy showed no striking differences with the exception of age; treatment failure and particularly death was mainly observed in patients older than 50 years. The presence of HBe antigen did not discriminate the two groups; absence of HBe antigen was only found in patients responding to prednisone (Table 4).

Additional information can be obtained from studying the effect of prednisone on SGOT-levels. In nearly all patients with SGOT-levels $5 \times$ the upper limit of normal, treatment with 30-60 mg of prednisone induced a marked drop in SGOT-levels within two weeks. When the dose of prednisone was tapered

TABLE 4

Treatment responses to prednisone in HBsAg pos severe CALD - Comparison of initial features

	<i>Failure</i>	<i>Response</i>
age, > 50 yrs	4 (4 ⁺)	2
50 yrs	3 (0 ⁺)	6
HBe: positive	4	5
negative	0	3

down to 10-20 mg of prednisone at 6 weeks, SGOT-levels rose again in about 50% of patients (4). In the latter group higher doses of prednisone are needed to suppress disease activity; the benefit-risk ratio of high dose prednisone treatment in these patients is thus unknown.

SUMMARY

HBsAg positive chronic active hepatitis is different from HBsAg negative hepatitis in presenting features, and response to prednisone treatment. Since current knowledge on effects of corticosteroid treatment in HBsAg positive chronic active hepatitis is based on less than 20 patients, additional data on prednisone versus control therapy in HBsAg positive chronic active hepatitis are urgently needed. Based on available data, we advocate prednisone treatment in HBsAg positive chronic active hepatitis only when the disease activity is severe.

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Alcoholic cirrhosis does not necessarily follow alcoholic hepatitis

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Alcoholic liver injury has three main manifestations: 1) steatosis, the pathogenesis of which is well established, 2) hepatitis, initially predominating in the center of the lobule or in zone 3 of the acinus, but extending to the peripheral zone and the portal tracts, and 3) fibrosis, progressing to cirrhosis, initially micronodular. In the older literature, a transition of fatty liver to cirrhosis was generally assumed and also extended to nutritional deficiencies. In the last 15 years, however, alcoholic hepatitis (1), defined clinically by hepatomegaly, anorexia, abdominal distress and leukocytosis, with or without jaundice, was considered a necessary precursor for cirrhosis in alcoholics. Histologically it is characterized by alcoholic hyalin and by swelling of the hepatocytes progressing to necrosis and associated with accumulation of leukocytes within the parenchyma. Recent observations have incriminated an alteration of constituents of the cytoskeleton, the microtubules, in both the formation of alcoholic hyalin (consisting of intermediate filaments) (2, 3) and the hydropic swelling of the hepatocytes. Proteins excreted by the normal hepatocytes, such as albumin and transferin, are retained (4) and cause swelling.

The thesis to be presented here, based on both biochemical studies (5) and investigation of baboons on an adequate diet which Dr. Lieber had exposed to ethanol for up to four years (6), suggest that fibrosis in alcoholics may terminate in cirrhosis without significant hepatitis. This raises the question as to the stimuli for the excess formation of connective-tissue fibers leading to cirrhosis. The most effective stimulus is necrosis of hepatocytes associated with inflammation as a direct effect of alcohol on the cell or as secondary result of the fibrosis, interfering with cellular nutrition. Whether immunologic processes, incriminated in alcoholic liver injury (7), cause fibrosis, is not established. However, evidence in experimental animals indicated that ethanol has a direct fibroblastic effect, exerted either by excess lactate resulting from the oxidation of alcohol or by acetaldehyde formed from ethanol. This is demonstrated by established parameters of fibroplasia found before hepatocellular necrosis is noted. This includes an increase of hepatic hydroxyproline, the amino acid characteristic of collagen,

increased activity of prolyl hydroxylase, the enzyme forming hydroxyproline from proline, and increased incorporation of the radioactive precursor proline into collagen. Moreover, urinary hydroxyproline is raised and activities of collagenase are under investigation. These alterations have been confirmed in man in various stages of alcoholic liver disease, including pure steatosis. Hepatic hydroxyproline and glucosaminoglycans as components of the connective-tissue matrix are elevated, urinary hydroxyprolines are raised and liver cell cultures of alcoholics show increased transformation of proline to hydroxyproline. These parameters are particularly high in alcoholic hepatitis, when necroinflammation complicates the effect of ethanol and steatosis; they are much higher than in chronic active hepatitis. Moreover, in alcoholic liver disease, an early excess of the hard variety of collagen, now designated as type I, causes portal hypertension before cirrhosis sets in, as result of central hyaline sclerosis (8). Moreover, in nutritional steatosis in rats without liver cell necrosis, hepatic hydroxyproline and hexosamines are elevated and incorporation of proline into hydroxyproline is enhanced.

The biochemical observations as to a fibroblastic stimulus by ethanol and also by steatosis agree with histologic features in Dr. Lieber's baboons, in which initially a steatosis of both small and large-droplet type develops associated with an increase of the reticulum framework. Eventually fat granulomas with deposition of diastase-resistant PAS-positive granules form from excess fat in hepatocytes. Fibrosis around these granulomas leads to the formation of septa linking the granulomas in the absence of leukocytes, and hepatocytes disintegrate from encircling fibrosis. After two years Dr. Lieber's baboons show frank cirrhosis in which now, presumably secondary, inflammatory foci develop in the septa.

In steatotic livers of human alcoholics, similar fat granulomas and connecting septa consisting first of reticulin (collagen, type III) and then of collagen I can be observed, again in the absence of leukocytes. Moreover, Dr. Elias and I (10) demonstrated in three-dimensional reconstructions the transformation of the human fatty liver into cirrhosis by the linking of septa radiating from the central canals and the portal tracts and developing within the lobular parenchyma.

Evidence thus exists for fibrosis with transition to cirrhosis without significant primary alcohol-induced necroinflammation in alcoholic steatosis. This does not exclude fat granulomas or secondary necrosis of hepatocytes from encircling fibrosis and late development of inflammatory foci in the cirrhotic stage. This evidence is based on the sequences in the baboons of Dr. Lieber, from the study of histologic sequences as well as from reconstructions in man, though, obviously, transient unrecognized and asymptomatic hepatitis may have been missed. Finally, clinical observations of development of cirrhosis without

hepatitis have been reported repeatedly, in part supported by biopsy. An example is cirrhosis in Sake drinkers in Japan (11), in whom both alcoholic hyalin as well as leukocytic inflammation is hardly ever encountered.

The listed observations, by necessity presented rather briefly, permit several conclusions. Cirrhosis as the end result of fibrosis in alcoholics may develop not only from alcohol-induced hepatocellular necrosis and associated inflammation, reflected in a clinically and morphologically recognized alcoholic hepatitis, but also by a fibroblastic stimulation from ethanol and in part from the steatosis. The relative contribution of each factor in the individual alcoholic patient might possibly depend on amount of alcohol consumed and the drinking pattern. The conclusion that cirrhosis may develop in alcoholics asymptotically and in a creeping fashion, has several implications. Suspicion of this transition is justified even in the absence of obvious clinical or laboratory manifestations. It may be better recognized by chemical and histologic parameters of fibroplasia than by the common hepatic tests, which mostly reflect liver cell damage. The clinical application of the chemical parameters of hepatic fibroplasia are at present being investigated. Similarly, a specific antifibroblastic therapy may be promising, which at present is being developed. The increased turnover of the connective tissue in the fibrosing liver, as well as the several steps in the synthesis of the connective tissue substances, are important in this promising therapeutic modality of the future. To what degree these considerations might apply to cirrhosis of etiologies other than alcoholic, remains still in the realm of speculation.

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Does alcoholic cirrhosis follow exclusively alcoholic hepatitis?

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Due to the peculiarities of the ethanol metabolism, liver damage, visible in the light microscope, occurs only after chronic, i.e. daily, intake of alcohol. While steatosis arises in a few weeks and disappears in the same space of time when alcohol consumption is discontinued, alcoholic cirrhosis develops only after many years of chronic alcoholism (Fig. 1). According to a recent statistic survey

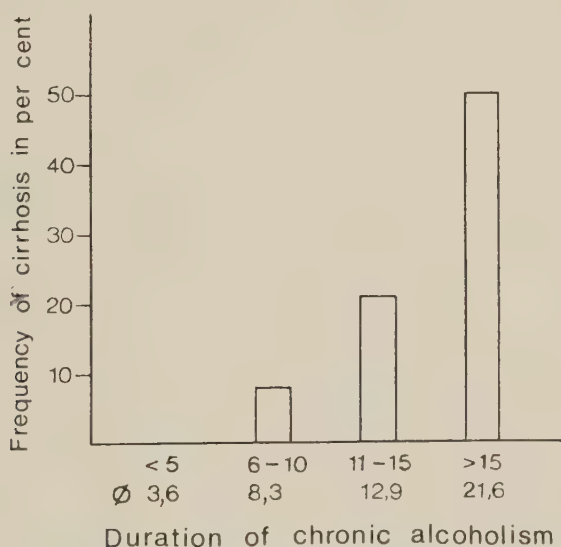


FIG. 1. Relation of the frequency of cirrhosis to the duration of chronic alcoholism. After Thaler, H. (3).

cirrhosis may already originate from a daily intake of more than 60 g of pure alcohol in males and more than 20 g in females (2, 3). With increasing doses cirrhosis morbidity rises dramatically (Fig. 2).

Alcoholism is a main indication for liver biopsy. In our material (Table 1), among 22995 bioptically investigated patients 4060 were chronic alcoholics (17.7%). 2108 or 51.9% of them showed fatty liver. Alcoholic hepatitis was

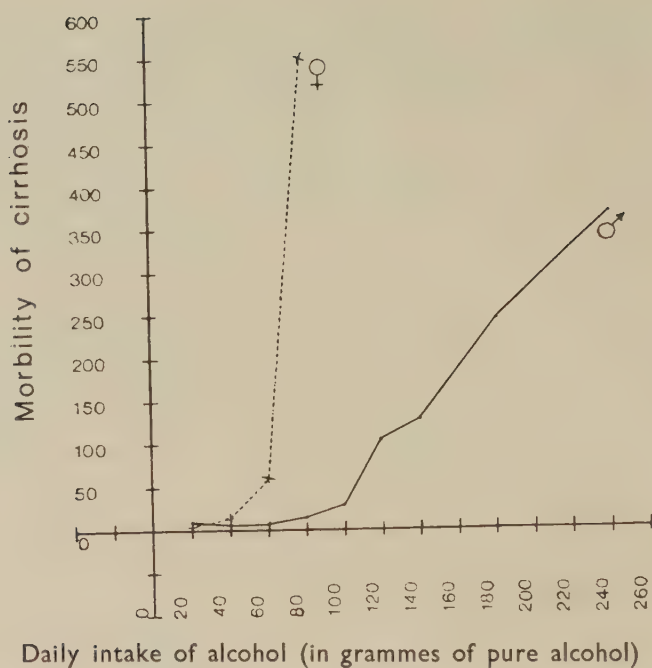


FIG. 2. Relation of the morbidity of cirrhosis to the daily intake of alcohol in males and females. After Thaler, H. (3).

TABLE 1

Morphologic findings in chronic alcoholics

	No.	%
Fatty liver	1874	46.2
Fatty liver with fibrosis	234	5.8
	2108	51.9
Alcoholic hepatitis	675	16.6
Active alcoholic cirrhosis	579	14.3
Hepatocellular carcinoma in active alcoholic cirrhosis	2	0.0
	1256	30.9
Centrilobular sclerosis after alcoholic hepatitis	227	5.6
Inactive alcoholic cirrhosis	326	8.0
Hepatocellular carcinoma in inactive alcoholic cirrhosis	20	0.5
Normal liver	34	0.8
Insufficient material	89	2.2
	4060	100.0

diagnosed in 1256 patients (30.9%). 675 of them presented alcoholic hepatitis with various degrees of fibrosis but with preserved lobular architecture. In 579 drinkers there was already an active alcoholic cirrhosis seen, i.e. a nodular umbau of the liver together with the histological features of alcoholic hepatitis. The frequency of some important characteristics of alcoholic hepatitis, observed in our material, is given in Table 2. It seems to be quite probable that there are geographical differences in this respect.

TABLE 2

Histologic findings in 675 cases of non-cirrhotic alcoholic hepatitis

	No.	%
Steatosis	663	98*
Mallory bodies	615	91
Polymorphonuclear infiltrates	168	25
Siderosis	297	44
Necrosis with fibrosis:		
Centrilobular	539	80
Centrilobular and periportal	41	6
None	95	14**

* Alcoholic hepatitis without steatosis was only seen when patients for three weeks or longer abstained from drinking alcohol.

** Early cases of alcoholic hepatitis.

Serial biopsies were performed in 54 patients up to twelve per individual case. In 16 alcoholics, who were not able to stop their alcohol-abuse, the development from alcoholic hepatitis to alcoholic cirrhosis could be studied. On the other hand, in 19 other patients, who abstained completely from alcohol, healing of the alcoholic hepatitis with complete restitution or with centrilobular sclerosis (so-called central hyalin necrosis) could be noted (Table 3).

In 227 patients (5.6%), who did reduce their alcohol intake considerably or abstained from drinking before biopsy was performed, no alcoholic hepatitis but centrilobular sclerosis with or without various degrees of fatty changes were found. In 326 patients, former chronic alcoholics, (8%), biopsy revealed inactive cirrhosis. 34 persons, who did not drink for the past three weeks or longer, had normal livers.

Popper (2) points out that there is a second, slower process of deformation in livers of alcoholics, due to the fibrogenic effect of alcohol. Actually, mild fibrosis may be seen in alcoholic fatty livers which is obviously not caused by a foregoing alcoholic hepatitis. It consists of a centrilobular pericellular collagenisation (chicken wire-fibrosis) and/or fine porto-portal fibrous septa which

TABLE 3

Outcome of alcoholic hepatitis in relation to alcohol intake, studied by repeated liver biopsies

	<i>Alcohol intake</i>	
	<i>stopped</i>	<i>continued</i>
Complete restitution	1	—
Centrilobular sclerosis	18	—
Alcoholic hepatitis	—	16
Alcoholic cirrhosis:		
active	—	16**
inactive	4*	—
Total	23	32

* Stopped drinking only after development of cirrhosis. Including one case of hepatocellular carcinoma.

** Including two cases of hepatocellular carcinoma.

may be complete or uncomplete. We observed this collagenisation in 234 cases, 5.8% of all chronic alcoholics or 11.1% of the alcoholic fatty livers. Eight of these cases were controlled by serial biopsies. Up to four biopsies were performed. During a maximum control period of four years no increase of collagenisation was noted in spite of continuing abuse of alcohol.

From these findings it is concluded that alcoholic hepatitis is the usual and probably the inevitable precursor of alcoholic cirrhosis. Theoretically, it cannot be denied with certainty that alcoholic cirrhosis may also arise from simple collagenisation in alcoholic fatty livers. Though the possibility of such an event cannot be excluded, the event certainly is very rare.

SUMMARY

4060 chronic alcoholics were studied by liver biopsies. Alcoholic cirrhosis originated exclusively from alcoholic hepatitis. In alcoholic fatty livers a collagenisation without foregoing alcoholic hepatitis was observed but no transition of this lesion to cirrhosis was seen.

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SYMPOSIA
PRIMARY AND SECONDARY
IMMUNOLOGICAL DEFICIENCIES

Primary and secondary immunological deficiencies

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Let me introduce this Symposium on immunological deficiencies with a picture (Fig. 1) taken from a paper published in 1939—almost 40 years ago—by Tiselius and Kabat (8). The white electrophoretic diagram with a markedly increased γ -globulin gradient is from a serum of a rabbit immunized with egg-albumin. The black one with a small γ -globulin gradient only is from the same serum after absorption with the antigen.

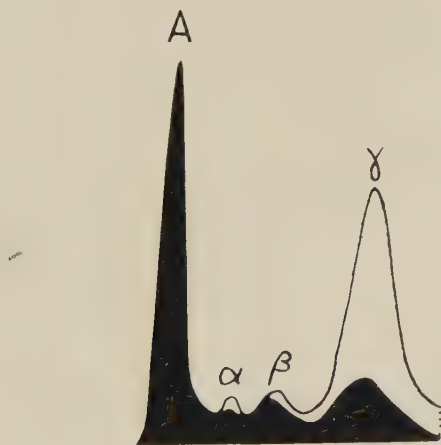


FIG. 1. a) *White diagram*: Electrophoretic diagram of the serum of a rabbit immunized with egg-albumin with markedly increased γ -globulin gradient; b) *Black diagram*: Electrophoretic diagram of the serum after absorption with the antigen (normal γ -globulin gradient) (8).

This was the first time that antibodies were related to the slowest of the 5 electrophoretic serumprotein fractions described by Tiselius and named with the third letter of the greek alphabet. For the first time the term γ -globulin, later replaced by *Immunoglobulin* entered into the field of immunology.

A fundamental source of new knowledge was then the application of the electrophoretic fractionation in clinical medicine which offers two models of disturbance of antibody-production: 1) agammaglobulinemia [Bruton (2)], 2) pa-

raproteinemic diseases (myeloma and macroglobulinemia). The patients affected with these immunoproliferative disorders supplied the chemists with heavy quantities of an homogeneous and easily separable fraction of the serum, which possesses the chemical structure of the immunoglobulins or antibodies respectively.

Before 1960 the analysis of these substances was limited to physicochemical characterization. By means of immunochemical methods—the double diffusion technique by Ouchterlony (1948) and the immunoelectrophoresis developed by

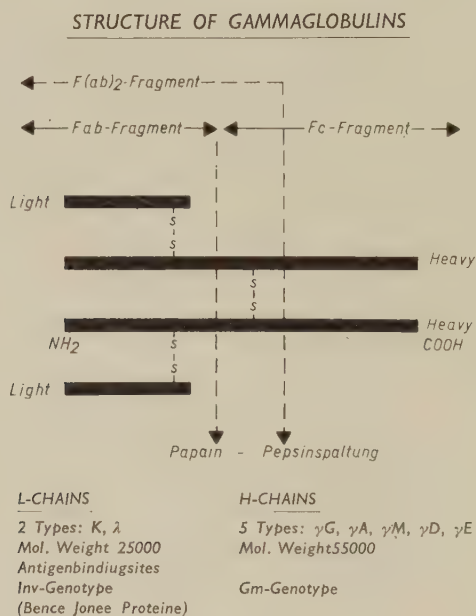


FIG. 2. Four-chain model of the immunoglobulin molecule.

Graber and Williams (1952) both a plurality and fragments (Bence-Jones proteins) of immunoglobulins were detected.

I want you to remember there only one of the major performances in this field: the monograph published in 1960 by Heremans: "Les globulines sériques du système gamma", where he described IgA for the first time (4). This gives me the opportunity to honour this meritorious member of the community of physicians, who dedicated and dedicate their scientific work to the progress of modern immunology.

The two clinical models just mentioned were of great importance not only from the humoral point of view, they also allowed to localize the immunoglo-

bulins on the plasma cells. These cells are either lacking in agammaglobulinemia or they do proliferate enormously in paraproteinemia.

However, it is surprising that such a long time had to elapse before the central cellular element in immunology, the *lymphocyte*, was adequately appreciated. Not until the early 1960s it was demonstrated that the small lymphocyte is a plasma-cell precursor.

Since 1960 there is an exponential growth of advances in immunological research.

On the humoral side the start was given by the elaboration of the four-chain model of the immunoglobulin molecule by Porter and Edelman (Fig. 2).

This model could be confirmed even by electron microscopy.

In the decade 1965-1974 data on aminoacid sequences of immunoglobulins increased explosively from zero up to more than 12,000 aminoacid-residues [Putnam (6)].

It seems to me, that nothing can demonstrate better the tremendous advances in this field than comparing figure 1 with the next one (Fig. 3) which represents the cristallographic structure of the variable domain of a rabbit antibody.

On the cellular side not only the central role of the lymphocyte was recognized. We also learned that there are more than one lymphocyte.

But not B and T lymphocytes only, also a lot of subpopulations like T-helper, -suppressor, -killer and memory cells have been distinguished. Beyond this the knowledge concerning the mediator system in immunology, which involves the complement system, the non specific cells (namely the phagocytes and macrophages), the lymphokines, the transferfactor increased rapidly -not to mention the immunogenetics, the evolution of immunoglobulins and some other fascinating topics.

These enormous advances are of great significance for the clinician who can obtain a better insight into the immunological disturbances, a more satisfactory classification of immunoproliferative disorders and a better understanding and more efficient methods for the treatment of the *experimenta naturae*, which are the topic of this symposium: the *immunodeficiency syndromes*.

Coming from Switzerland I would like to remember here that in 1951, one year before the classical paper by Bruton (2), Löffler in Zurich (5) reported on a patient with chronic lymphatic leukemia who had many episodes of bacterial meningitis and in whose serum γ -globulins were lacking, and that even earlier in 1950 Glanzmann and Riniker in Berne (3) described a new disease which they called "essentielle Lymphozytopenie", which later became the "Swiss type of agammaglobulinemia".

No doubt however that Bruton (2) must be considered the father of the new chapter of clinical medicine entitled "immunological deficiency diseases".



FIG. 3. A hypothetical model of the V-domain of a rabbit anti-pneumococcal antibody built by J.-C. Jaton, with the aid of E. Padlan and D. Davies (NIH, Bethesda), on the basis of the known X-ray crystallographic structure of the Fab fragment of MOPC 603 protein together with the amino acid sequence of the antibody. Photography by M. Pazdera, composition by H. Stahlberger. Published in "Basel Institute for Immunology, Annual Report 1976" (cover).

We can now leave the history and come back to clinic, which is the main purpose of this Congress. Let me conclude this introduction with the following Figure 4.

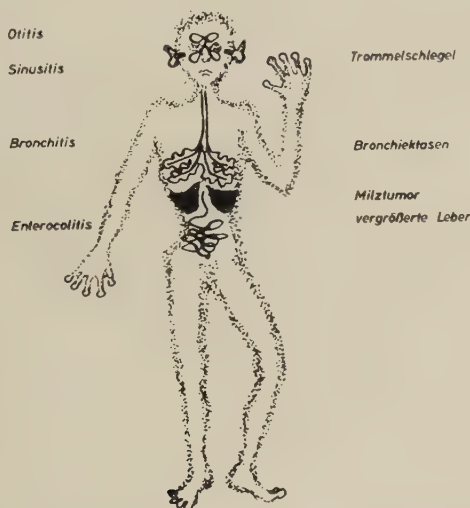


FIG. 4. Artistic summary of the clinical picture of antibody deficiency syndrome (designed by a patient with agammaglobulinemia) (7).

It was designed by one of our patients with agammaglobulinemia, who is a wellknown painter in Geneva. It is the artistic summary of the clinical picture of what Barandun (1) in our clinic named antibodydeficiency syndrome with sinusitis, otitis, bronchitis, enteritis, hepatosplenomegaly and clubbing of fingers and toes.

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Disorders of phagocyte function

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It is well known that *numerical* deficiency of phagocytic cells in the peripheral blood as in aplastic anemia or leukemia is a common cause of bacterial and fungal infection. However, during recent years it has been demonstrated that patients may suffer from recurrent infections due to *functional* defects in phagocytosis even when peripheral neutrophil or monocyte counts are normal. In reviewing the functional capacity of the phagocytes it is convenient to consider three interrelated phases: firstly, the chemotaxis or the directed movement of the cells to the sites of inflammation which is particularly accelerated by the complement components C_3 , C_5 and the complex of C_5 , 6 , 7 . Secondly, the phagocytosis or ingestion of microorganisms or other antigens, and thirdly, the cellular and metabolic factors involved in the killing of microorganisms.

During recent years, three basic types of abnormalities of leukocyte chemotaxis have been describe: 1) intrinsic defects of the leukocytes, 2) abnormalities of chemotactic factor production, particularly deficiency of complement factors, and 3) inhibitors of chemotaxis that act either directly on the leukocytes or by antagonizing the action of the chemotactic factors (Clark, 1978; Quie and Cates, 1978; Snyderman and Pike, 1977; Stossel, 1974). Defective leukocyte chemotaxis due to cellular defects has been described in several clinical conditions with enhanced susceptibility to bacterial infections including uncontrolled diabetes mellitus, measles, influenza, severe burns, chronic renal failure, malnourishment, severe bacterial infections and graft-versus-host disease. Usually, these are transient defects. Permanent chemotactic defects have been demonstrated in congenital ichthyosis, hypogammaglobulinemia, rheumatoid arthritis, hyperimmune globulinemia E, the Chediak-Higashi syndrome (CHS), mannosidosis and some other conditions. It should, however, be mentioned that the first clinical report of a primary defect in neutrophil motility as a cause of recurrent infection occurred under the appealing title of "lazy leukocyte syndrome". So also in the kingdom of phagocytes lazy elements occur.

Patients with the CHS have several functional abnormalities of their neutrophils including impaired chemotaxis and lysosomal degranulation which may result from defective microtubular function. The diagnosis of CHS is made by identification of leukocytes with giant lysosomal granules, and the chemotactic defect has been postulated to result from the giant granules preventing

the neutrophils from squeezing through small spaces. Recent evidence, however, suggests that there may be a metabolic defect in CHS leukocytes as well. Very high levels of cyclic AMP have been demonstrated in these leukocytes, and treatment with ascorbic acid has brought about a sharp reduction in the levels of leukocyte cyclic AMP followed by reversal of defects in chemotaxis and lysosomal degranulation. Agents known to increase cyclic AMP levels seem to depress chemotaxis whereas those that increase cyclic GMP levels stimulate chemotaxis.

The mechanics of action of cyclic nucleotides in leukocytes is not well known. However, microfilamentous structures seem necessary for random migration i.e. non-directional cell migration while the microtubules have a cytoskeletal function and may be required to maintain the polarity of the cell in directed locomotion i.e. chemotaxis (Gallin *et al.*, 1978). Degranulation seems also partially mediated by the microtubules. Cyclic GMP or cholinergic agents seem to promote the assembly of tubular proteins to microtubules while cyclic AMP decreases this function. It is not surprising, therefore, that agents like ascorbic acid which offset the effect of elevated cyclic AMP, stimulate neutrophil motility and degranulation in CHS leukocytes.

Accumulation of inflammatory cells at the sites of antigenic challenge depends also upon adequate production of chemotactic factors, and many chemical mediators have been described as having activity *in vitro* but most of these have not yet been proven to possess biological activity *in vivo*. However, a great deal of evidence suggests that activation of the complement system with production of C_5 cleavage products (C_5a) results in potent chemotactic activity for both leukocytes and macrophages and it has been demonstrated that absence of C_5 or hypercatabolism of C_3 (lack of C_3b inactivator) result in severe infections. A definite relationship between susceptibility to infection and deficiency of other complement components (C_2 , C_{1r} , C_4 and C_7) has been difficult to establish. However, absence of other complement components may be associated with other diseases i.e. systemic lupus erythematosus, and diabetes mellitus.

The third mechanism of potentially defective chemotaxis involves serum factors that either antagonize the action of the chemotactic factors or act directly on the phagocyte. Chemotactic factor inactivators are present in normal serum and destroy the biologic activity of a variety of chemotactic factors by enzymatic cleavage. Excessive serum levels of these inactivators have been detected in several disorders associated with cutaneous anergy, including Hodgkin's disease, lepromatous leprosy, and sarcoidosis. Clinically, these patients have enhanced susceptibility to bacterial, viral, and fungal infections, and it is likely that the combination of multiple intrinsic leukocyte defects as well as excessive circulating inflammatory inhibitors contribute to this increased susceptibility. Patients with alcoholic liver disease also have increased susceptibility

to serious bacterial infections and this may be related to excessive levels of chemotactic factor inactivators, to cell-directed inhibitors or both. Direct cellular inhibition of chemotaxis has also been described in the Wiskott-Aldrich syndrome, in patients with elevated levels of IgA and in cancer patients. In patients with the Wiskott-Aldrich syndrome high plasma levels of lymphocyte-derived chemotactic factor have been demonstrated, and it is postulated that constant exposure of neutrophils and monocytes to these high levels may "deactivate" the cells and render them less able to respond to a chemotactic stimulus.

The second phase of phagocyte function constitutes the ingestion of the antigens i.e. microorganisms and other particulate matter. Most microorganisms pathogenic to humans require specific antibody (IgG and IgM) directed toward the microbial surface components or heat labile serum factors (complement) before attachment of the microorganism to the phagocyte can take place (Figure 1). Both granulocytes and monocytes have receptors for IgG and heat labile factors on their surface. The Fab fragment of the IgG molecule is the part that

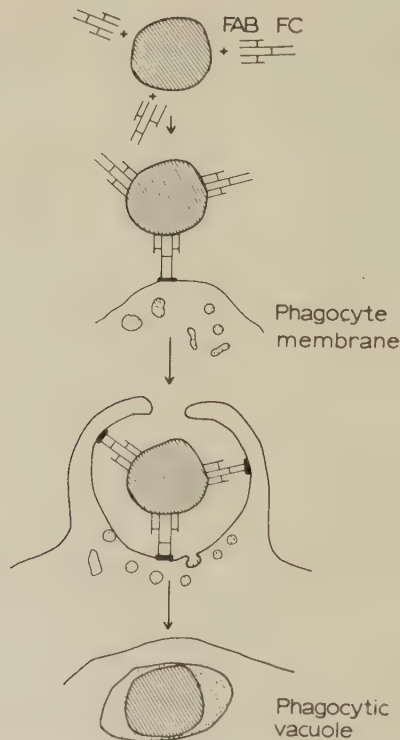


FIG. 1. Brief presentation of opsonisation, phagocytosis, intracellular killing and digestion of bacteria. See text.

attaches to the antigen and the attachment to the phagocyte is mediated by the Fc fragment. When attachment of the microorganism to the phagocyte occurs, the phagocyte membrane immediately surrounds the pathogen and a phagocytic vacuole is formed. The cytoplasmic granules or lysosomes which contain the digestive enzymes move toward the vacuole, fuse with the vacuole membrane and discharge their enzymes into the vacuole for rapid killing and digestion of the microorganisms to occur.

Disorders of the second step of the phagocytic process may be due to low concentrations of specific antibodies as in various types of hypogammaglobulinemia or in some rare instances to lack of complement factor C₅. Treatment of these patients with gammaglobulin or complement factors dramatically improves the patients' condition.

The efforts of the phagocytic cells are crowned by the destruction of the ingested microorganism. During recent years, several disease syndromes characterized by chronic bacterial and fungal infections have been related to defects in intracellular killing of the microorganisms (Babior, 1978; Niethammer *et al.*, 1975; Quie, 1975). Chronic granulomatous disease (CGD) remains the prototype of this sort of defect. Patients with this disease usually develop infections during their first months of life. The earliest lesion are eczematoid dermatitis around the ears, nose and mouth progressing to purulent skin lesions with local suppurative lymphadenitis. Indolent infections of the liver and internal organs frequently occur. Healing is slow and purulent drainage from the lesions for weeks is characteristic. The microbial species causing disease include several catalase-producing bacteria and fungi such as staphylococci, gram-negative bacilli, aspergillus and candida, while infections with pneumococci and streptococci which produce hydrogen peroxide, are rare. When granulocytes from normal individuals are incubated with f.ex. *Staphylococcus aureus* and serum at 37°C, a marked reduction in the number of viable bacteria is observed and very few bacteria within the leukocytes survive (Figure 2). In contrast, when granulocytes from patients with CGD are incubated with *Staph. aureus* and serum, only minor reduction in viable bacteria can be demonstrated and large numbers of bacteria survive within the leukocytes, indicating normal phagocytosis but markedly impaired bactericidal activity of the granulocytes. This has also been demonstrated for monocytes. The findings are supported by the results of biochemical studies. Glycolysis which is the energy source for phagocytosis, is normal. But phagocytosis does not result in increased oxygen uptake, hexose-monophosphate shunt activity or generation of reactive oxygen radicals (O₂, hydroxyl radical, singlet oxygen) and hydrogen peroxide or chemiluminescence characteristic for the intracellular killing of microorganisms. However, when oxygen radicals are provided by ingested microbes which produce hydrogen peroxide (i.e., strepto-

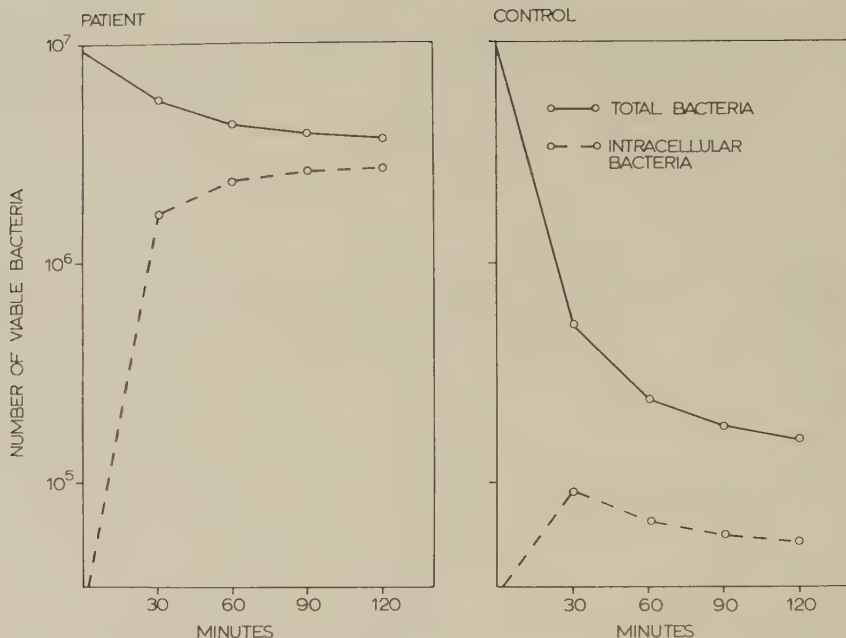


FIG. 2. Viable bacterial counts during incubation of *Staphylococcus aureus* with neutrophil granulocytes from a patient with chronic granulomatous disease and a normal control.

cocci or pneumococci) or by particle-associated oxidases, normal killing of bacteria occurs. The oxygen radical derived from *bacterial* hydrogen peroxide may, therefore, substitute for the lack of *phagocyte* oxygen radical in the phagocytic vacuoles and allow one of the strongest bactericidal systems of the phagocyte to work. This system is made up of myeloperoxidase, a halide and reactive oxygen radicals. Thus, hydrogen peroxide generating bacteria commit suicide within these phagocytes. Based upon these findings some improvement in the treatment of this condition have been made, and several of these patients have now reached the age when they have to be treated by the internists. Our oldest patient with CGD is now 28 years old and is full time working as a mechanic.

Chronic infections due to impaired killing of phagocytized bacteria may also result from complete absence of leukocyte myeloperoxidase or glucose-6-phosphate dehydrogenase and probably also from glutathione peroxidase deficiency. However, these are rare disorders and the reduced bactericidal activity of the granulocytes which may occur in patients with extensive burns and in some patients with severe bacterial infections like prolonged septicaemia or pneumonia seems more important as this may contribute to a fatal outcome in some of these patients (Solberg and Hellum, 1972). In the preleukemic state

of myeloblastic leukemia i.e. before the diagnosis can be made from clinical and morphological findings, reduced intracellular killing of phagocytized bacteria can be demonstrated (Solberg *et al.*, 1975). Based on results of phagocyte function studies, a presumptive diagnosis of leukemia can therefore be made before the disease is diagnosed from morphological examinations—sometimes several months before. Most likely, the enzyme deficiencies of the leukocytes in leukemia appear before the morphological changes can be demonstrated.

Most of the disorders of phagocyte function are rare conditions. However, these disorders have significantly improved our knowledge of the various steps involved in the phagocytic process and clearly demonstrated the importance of each step in antimicrobial host defence.

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Idiopathic and secondary immunoglobulin deficiencies. Clinical and immunological studies

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Patient with various forms of primary deficiencies of humoral immunity (HI) often present increased susceptibility to many types of infection and therefore show recurrent and chronic infections, mainly of the respiratory tract. A wide spectrum of clinical manifestation may occur in deficiencies of HI; many of them often occur in congenital hypogammaglobulinemia (Bruton's disease) and in the so called common variable immunodeficiency (CVI).

It may take up to two years after birth until the Ig production is adequate but if panhypogammaglobulinemia persists beyond 18-24 months there is a permanent defect, not just transient hypogammaglobulinemia of infancy.

It is now well known that antibody deficiencies may occur at any age and are not confined to the pediatric age. They are found rather frequently as a secondary phenomenon in various diseases, particularly the lymphoid malignancies (Bonomo *et al.*, 1974).

The incidence of various ID in Italy (Italian Registry of ID by Aiuti, Giunchi and others) shows a high frequency of defects of humoral immunity particularly CVI and IgA deficiency (Table 1).

Defects of HI may produce complete agammaglobulinemia (Bruton's type X-linked agammaglobulinemia) while selective deficiencies may affect one or more of the major Ig classes and subclasses, even with isolated defects of synthesis of either K or λ light chains.

In typical cases of agammaglobulinemia infections dominate the clinical picture and are the terminal event; Ig's are markedly reduced or undetectable while tests of CMI are normal.

IgA deficiency appears to be the most frequent defect of humoral immunity: actually it occurs in about one out of 700 healthy adults (Table 2).

Many patients with isolated IgA deficiency suffer from recurrent polymucositis (upper and lower respiratory tract infections, chronic bronchitis, asthma, conjunctivitis, urethritis) and atopic eczema in infants, various cases of g.i. disorders, some with coeliac disease (Table 3).

TABLE 1

Primary immunodeficiency syndromes, relative distribution of 538 cases (National Registry, Aiuti, Giunchi *et al.*, 1978)

	<i>No. of cases</i>	<i>%</i>
Severe combined immunodeficiency (CID)	30	6.09
CID with purine dysfunction	4	0.81
Di geore syndrome	3	0.61
Nezelof's syndrome	47	9.55
Bruton type agammaglobulinemia	21	4.27
X-linked agammaglobulinemia with yper-IgM	5	1.02
Common variable hypogammaglobulinemia	69	14.02
Selective IgA deficiency	223	45.32
Selective IgM deficiency	12	2.44
ID with short-limbed dwarfism	6	1.22
Ataxia-teleangiectasia	29	5.89
Wiskott-aldrich syndrome	13	2.64
Defects of complement fractions	4	0.81
Defects of chemotaxis	2	0.41
Defects of phagocytosis	19	3.86
Chronic granulomatous disease	5	1.02
Other	158	— —
TOTAL	650	

TABLE 2

Results of testing sera from blood donors and patients for absence of IgA

<i>Group</i>	<i>No. tested</i>	<i>No. with IgA deficiency (< 0.05 mg/ml)</i>	<i>Incidence</i>
1. Blood donors	12,203	16	1:762
2. Patients (miscellaneous disease)	1,320	2	1:660
3. Others	—	25	—

Various patterns, a few of them suspected of autoimmune nature (RA, idiopathic thrombocytopenic purpura) are associated with IgA deficiency.

IgE levels were more frequently raised in our IgA deficient patients with respiratory tract disease than in those without (Table 4).

The frequent occurrence also in our series of precipitin against cow's milk and the serum of other ruminants (Fig. 1) suggests a role of immune exclusion for secretory IgA in preventing a systemic immune response to food antigens,

**PRECIPITATING ANTIBODIES AGAINST MILK, HORSE AND RUMINANT SERA
IN SELECTIVE IgA DEFICIENCY**

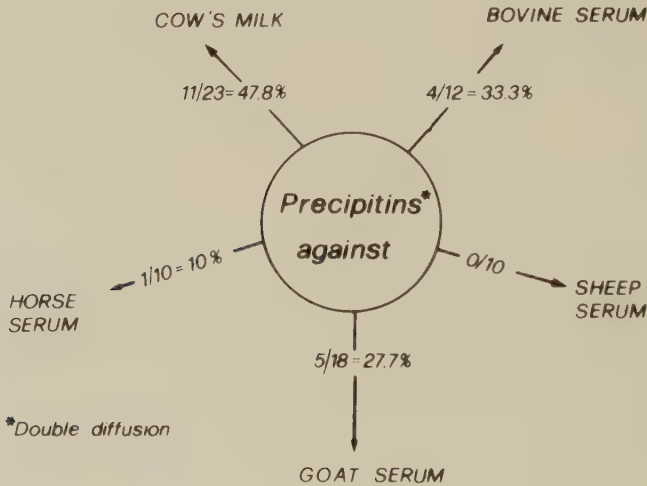


FIG. 1

TABLE 3

Selective IgA deficiency - Personal observations

<i>Clinical features</i>	<i>No. of cases</i>
B.D., asymptomatic	8
B.D., rhinopharyngitis and/or sinusitis	10
B.D., diarrhea	9
B.D., polycystic kidneys hypertension	1
Rheumatoid arthritis	2
Recurrent infections	8
Celiac disease	5
Chronic persistent hepatitis	6
Follicular lymphoma	1
Idiopathic thrombocytopenic Purpura	1
Mental retardation and seizures	2
Asthma	2
Eczema	2
TOTAL	59

B.D. = Blood Donor

TABLE 4
Selective IgA deficiency. Relationships between serum IgE levels and respiratory tract disease

		Serum IgE levels	
		> 7 IU/ml	< 7 IU/ml
IgA-deficient subjects	With respiratory tract disease	9	1
	Without respiratory tract disease	3	9

possibly by reacting with, and so blocking, potential antigens in the gut (Dammacco *et al.*, 1976).

IgA deficient patients have often serum antibodies to IgA; this explains the risk of anaphylactic reactions, severe in a few cases, to blood transfusion and gamma globulin injections (which contain about 5 mg/ml of IgA). However, if chest infections are frequent they clearly benefit from regular γ -globulin injections.

Anyway washed red blood cells should be given to IgA deficient patients, with particular attention to treatment with γ globulin for the risk of severe anaphylactic reactions.

Patients with CVI characteristically have severe hypogammaglobulinemia involving all Ig classes; the clinical pattern is dominated by respiratory and intestinal infections; malignancies are frequent.

Infant and adults, males and females, are affected; there is no familiar distribution and the number of circulating B lymphocytes is low or normal.

In most cases all Ig's are at very low levels; very often serum IgA are below recordable levels; and IgG and IgM are very low; a few have normal or raised levels of IgM. Several of these patients have defects also in CMI, although the number of circulating T cells is frequently normal.

In cases with various forms of primary humoral immune deficiencies (CVI, panhypogammaglobulinemia, etc.) the B lymphocytes generally fail to mature into plasma cells and to synthesize and secrete Ig's upon stimulation with the polyclonal B cell activator pokeweed mitogen.

However, it was shown recently (Wu *et al.*, 1973; Siegal *et al.*, 1978) that this differentiation was possible in CVI under ideal conditions *in vitro*, although the plasma cell production was quantitatively very deficient.

This differentiation was not demonstrable in Bruton's type and thymoma-associated hypogammaglobulinemia.

This deranged cellular differentiation could originate from a variety of defects, some intrinsic to the B cells, others due to extrinsic factors that modulate B cells, (suppressor or helper T cells, serum inhibitors, etc.) (Table 5).

Anyway no single abnormality can be expected to emerge as the cause of the various forms of deficiencies of HI.

TABLE 5

Heterogeneity of the pathogenetic factors in common variable immunodeficiency

Quantitatively poor plasma cell production ascribable to one or more of the following factors:

- | | |
|--------------------------------------|-----------------------------------|
| — Abnormalities in modulator T cells | — Intrinsic B cell defect |
| a) excessive suppressor activity | — Suppressive effect by monocytes |
| b) defective helper function | — Local suppressor factor |
| | — Others |

Complications in hypogammaglobulinemia

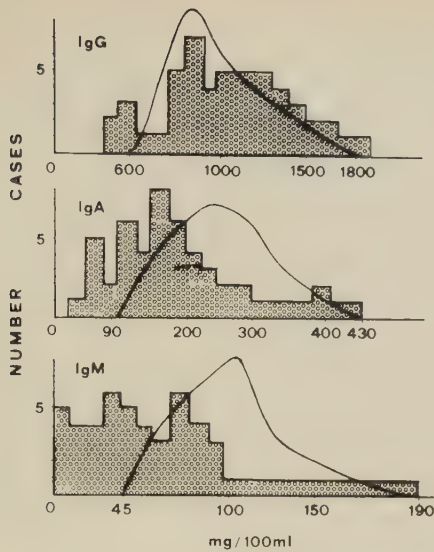
Bacterial infections (*Haemophilus influenzae*) are common manifestations of hypogammaglobulinemia. These patients tend to respond normally to viral infections; however, herpes zoster is rather frequent; vasculitis and chronic brain disease often with dermatomyositic features very likely caused by ECHO viruses occur in these patients. Other complications include arthritis, eczema, allergy to antibiotics. An increased incidence of leukemia and other solid tumours in sex-linked and variable ID has been reported as well as pernicious anemia, malabsorption from giardia infection or increase in small bowel bacteria, chronic cholangitis, cirrhosis.

Secondary immune deficiencies

Immunodeficiencies are being demonstrated in an ever increasing number of diseases. Catabolic mechanism (nephrosis), bone marrow disorders, various toxic factors (uremia, diabetes, thyrotoxicosis, cytotoxic therapy, etc.) and several lymphoproliferative diseases can be associated with hypogammaglobulinemia.

In our series leukemia (lymphoid), malabsorption, leprosy, glomerulonephrosis occurred associated with panhypogammaglobulinemia or with IgA-IgM deficiency; cases with cancer and Hodgkin's disease showed IgM deficiency.

Actually this latter disease, that is a classic demonstration of CMI deficiency with intact HI, often shows levels of IgM below the normal average (Fig. 2).



SERUM Ig LEVELS IN HODGKIN'S DISEASE

FIG. 2. Serum IgG, IgA and IgM levels in 54 patients with Hodgkin's disease, mostly stages III and VI (dotted regions). Normal ranges are indicated by the continuous line.

INFECTIONS IN MALIGNANT MONOCLONAL GAMMAPATHIES

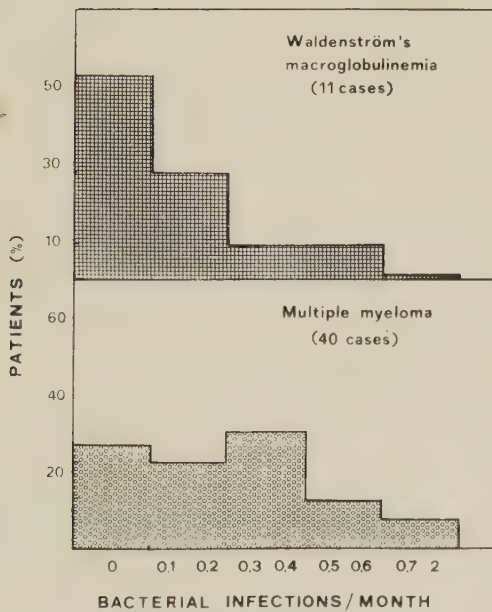


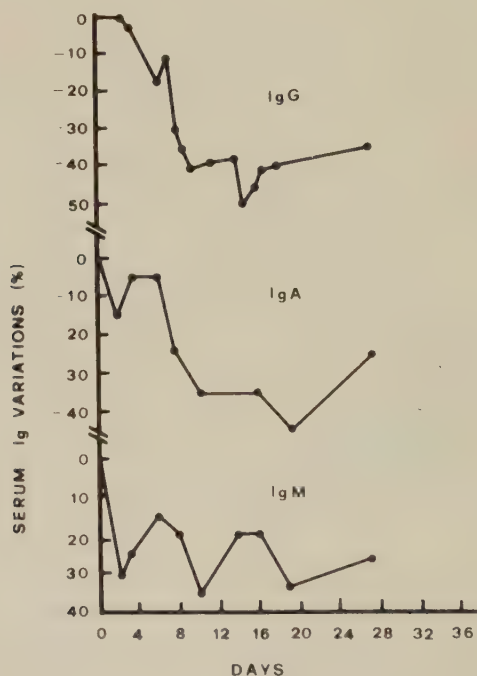
FIG. 3. Occurrence of respiratory tract infections in 40 patients with multiple myeloma and 11 patients with Waldenström's macroglobulinemia in a two-years' follow-up study.

Many acute infections are associated with a transient defect in CMI; tuberculosis, syphilis and leprosy all show defects in CMI. Recurrent infections from hypogammaglobulinemia are frequent in CLL.

Patients with monoclonal gammopathies, particularly MM, have a significant defect in the ability to produce specific antibody (70% of our cases) and many have low levels of the "normal" Ig's outside the M component. Pneumonia and septicemia are common complications; episodes of infections are more frequent in MM than in WM (Fig. 3).

Ig deficiency was often found in cases of malabsorption (in 7 out of 15 cases), most frequently of IgM (in 4 cases), sometimes of IgA.

Selective Ig's deficiencies (IgA + IgM, IgM) are more frequently associated to disease states than panhypogammaglobulinemia.



EFFECTS OF CORTICOSTEROIDS

(Methyl prednisolone 60 mg x 5 days)

ON SERUM Ig LEVELS

FIG. 4

Among the many drugs that interfere with immune competence the most obvious are those used for treating chronic inflammatory disorders and malignancy. In particular, corticosteroids depress CMI producing lymphopenia and also marked reductions of all Ig's levels (Fig. 4).

Among the many therapeutic procedures that may produce defects of immunity, radiation treatment of mammary cancer showed a marked depressive effect on *in vitro* lymphocyte transformation to PHA with decreased number of circulating T-lymphocytes.

In conclusion primary defects of HI may occur not only in infancy but are now often detected in adults, particularly in cases of selective defects of HI (absence of one or two Ig's more often IgA) and of CVI.

Defects of HI are often associated with various disease states, particularly lymphoproliferative diseases and chronic infections.

ABBREVIATIONS

CLL = chronic lymphocytic leukemia
CMI = cell mediated immunity
CVI = common variable immunodeficiency
HI = humoral immunity
ID = immunodeficiency
MM = Multiple myeloma
WM = Waldenström's macroglobulinemia

ABSTRACT

Primary defects of humoral immunity (HI) occur most frequently in infancy although selective defects of HI and common variable immunodeficiency (CVI) are now often detected in adults.

IgA deficiency is the most frequent defect of HI often associated with poly-mucositis, but also occurring in healthy adults.

Infections, often though not exclusively of bacterial nature and also malignancies (lymphoproliferative diseases) are the clinical pictures most frequently associated with defects of HI.

The peculiar aspects deriving from a personal series of cases are reported.

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Cellular immunity deficiency

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DEFINITION AND BACKGROUND

The term cellular immunity as opposed to humoral immunity is by now becoming outdated and replaced by the terms lymphocyte mediated immunity or even T-lymphocyte mediated immunity. This change reflects development. We know that delayed type hypersensitivity is mediated by cells, but we also know that the cells are lymphocytes, more precisely T-lymphocytes and even more precisely certain well-defined subclasses of T-lymphocytes. Cell-mediated immunity, therefore, is a complicated concept, and cell-mediated immunity deficiency (CMID) even more so.

Fig. 1. shows a schematic representation of the immune system and the T- and B-lymphocyte lines. The macrophage engulfs and digests the macromolecular antigenic information which is subsequently presented to antigen sensitive T- and B-lymphocytes. The specific interaction between antigen and lymphocytes stimulates lymphocyte proliferation. The T-lymphocyte line develops populations of T-helper lymphocytes, which help B-lymphocytes to start production of specific antibody and become plasma cells, T-memory lymphocytes, which represent immunological memory to previously experienced antigen, T-suppressor lymphocytes which regulate the immune system by suppression of selected functions and, T-effector lymphocytes which are able directly to interact with the specific antigen in antigen-containing tissue and thereby cause a delayed type, T-effector lymphocyte mediated type IV immune inflammation. The T-effector lymphocytes accomplish this through release of lymphokines (2, 13): Biologically active, lymphocyte-produced polypeptides, which are released from lymphocytes when they interact specifically with antigen.

CMID or T-lymphocyte deficiency is by definition a condition in which T-lymphocytes, which are developmentally and functionally dependent on the thymus, are absent or deficient at some level. The defect may involve all subpopulations of T-lymphocytes: Effector lymphocytes, helper-lymphocytes, memory-lymphocytes and suppressor-lymphocytes. Since the subject of my presentation is to describe T-lymphocyte deficiency, I am free to assume coexistence of an intact B-lymphocyte system, which, however, is seldom the case in clinical disorders.

ETHIOLOGY AND PATHOGENESIS

The defect of the T-lymphocytes may occur at different levels of their differentiation (3). For instance, the thymus and with it all T-lymphocytes may be congenitally absent; the T-progenitor lymphocytes may be absent or deficient; the thymic hormone or its effect may be deficient (16); the various T-lymphocyte subpopulations may be differently affected; and the ability of T-lymphocytes to produce lymphokines and similar mediators for recruitment of other cells and for induction of the inflammatory reaction may be deficient (15, 17).

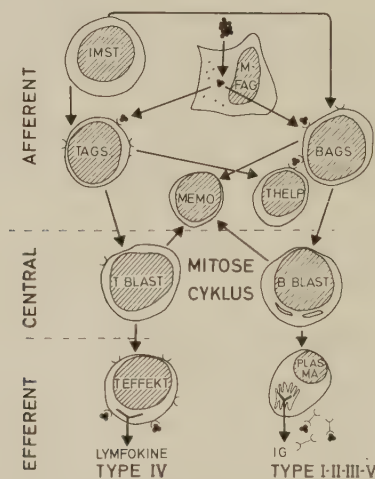


FIG. 1. Schematic representation of the immune system: IMST: Immunological stem cell; M-FAG: Macrophage; TAGS: Antigen sensitive T-lymphocyte; BAGS: Antigen sensitive B-lymphocyte; MEMO: memory cell; THELP: T-helper lymphocyte; T BLAST: Stimulated T-lymphocytes; B BLAST: Stimulated B-lymphocytes; T EFFEKT: T-effector lymphocytes; PLASMA: Plasma cells (G. Bendixen, *Cellular immunity deficiency*).

This background is necessary to understand the pathogenesis of deficiency disorders of the T-lymphocyte system. The various aspects of CMID can be described in the same way as other disease entities and will be briefly outlined below.

CMID is subclassified in two distinct types: The congenital types that are due to inborn errors of the T-lymphocyte system and become manifest in early life. Diseases of this group have served as interesting and informative models for our understanding of immunological deficiency and have initiated extensive scientific development and useful techniques for investigation and monitoring

of the immune system (3, 9, 12, 17). The experience from this field is now becoming valid in the exploration of the second major subclass of CMID: The acquired CMID, which is quantitatively much more important, common in large populations, not only confined to the pediatric field and importantly associated with several medical disorders (17). Some of the most well-known inborn T-lymphocyte deficiencies are indicated in Table 1. In these conditions the thy-

TABLE 1

Cell-mediated immunity deficiency (CMID)

Definition	Deficient function of T-lym-system
Ethiology	Inborn: seldom, interesting models. Acquired: frequent, insufficiently explored
Pathogenic levels	Thymic aplasia, lack of T-lym-progenitors, lack of thymic hormone, subpopulation defects, lymphokine defects
T-effector-lym deficiency	Insuff. inflamm. process. Essential for: Infection, contact eczema, autoimmunity, transplantation, malignancy
T-helper-lym deficiency	Insuff. ab.-production, apparently combined, but T-lym can restore
T-suppressor-lym deficiency	Deficient immune regulation. Exaggerated B-lym-response
T-memory-lym deficiency	Imm. memory quantitatively or qualitatively reduced or absent
Ethiology: inborn	Digeorge, Nezelof, candidiasis, others. Combined forms
Ethiology: acquired	Infection, hemopoietic disease, immune system disease, malnutritions, chronic disease, endocrinopathy, poisons, drugs, irradiation
Clinical signs	Infection, weak or absent inflammatory response, pharyngeal pouch malformation, empty paracortex in lfn., primary cause symptoms
Laboratory assays	Count lym, T/B, lym-transformation, LPS, PHA, Con-A, MLC, AG, lymphokines, several other assays
Treatment	Treat and prevent infection, remove cause, substitute

mus is absent or poorly developed, or there is a deficient differentiation and/or function of T-lymphocytes. Such disorders are often combined with defects in the B-lymphocyte system. Their developmental origin is evident from the association between thymic dysplasia and other signs of poor development of organs derived from the pharyngeal pouches, as for instance parathyroid or thyroid insufficiency or deformity of the face.

Acquired CMID has several etiologies (17): The T-lymphocyte system may be suppressed by infection, by diseases in the hemopoietic system or in the immune system itself, by malnutrition or certain chronic diseases such as uremia

or cancer, and in endocrine disorders; or it may be suppressed by poisons, immunosuppressive drugs, anti-lymphocyte globulin or by irradiation. Mostly we are without information on the detailed quality of these acquired T-cell deficiencies, and much clinical research work has to be done before the mechanisms behind acquired CMID are explained. However, in some cases information is available. For instance chronic mucocutaneous candidiasis is probably partly due to deficient lymphokine production of T-effector lymphocytes derived from an otherwise more or less normal T-lymphocyte system (15, 17). But the consequence of the deficiency is lacking capacity to eliminate candida infection in skin and mucous membranes, and the patient develops a serious invalidating disease.

It is well-known and an early observation, that virus infections may be associated with transient decrease or abolishment of CMID (6, 10, 11, 17). Familiar examples of this are for instance disappearance of the skin tuberculin reaction during morbilli or infectious mononucleosis. Animal experiments have shown (4) that this phenomenon is probably associated with any virus infection, and the suppression of the T-lymphocyte system seems much more pronounced and protracted than generally assumed. The effect may be due to interferon, which not only suppresses and eliminates virus but also the T-lymphocyte progenitors or the stem cells. The mechanism may very well explain the co-infection by virus and other microorganisms, which is familiar to most people working in the field of internal medicine. Alongside with the suppressive effect on the T-lymphocyte system, the hemopoietic system is also suppressed, and generation of all formed elements of the blood may be inhibited. There is generally good reason to associate acquired T-lymphocyte deficiencies with deficiency of the hemopoietic system. The mutual stem cell, the T-cell progenitors and the lymphocytes of the bone marrow have their origin, differentiation and early life course in close mixture with the hemopoietic cell system. Postinfectious anemia and postinfectious CMID may well be due to the same ethiological and pathogenetic mechanisms. Several diseases associated with CMID are associated with hematological deficiency as well, for instance uremia, cancer, malnutrition and endocrinopathies (17). It is adequate for the clinician to associate along these lines and suspect CMID in the management of clinical cases with hematologic disorders. It is less surprising that CMID accompanies diseases of the immune system itself, e.g. Hodgkin's disease or sarcoidosis (6, 17). One important chronic disease with CMID should be specially mentioned: In systemic lupus erythematosus (SLE) there is a clearly reduced T-lymphocyte function, probably due to anti-lymphocyte antibodies. The number of blood T-lymphocytes is considerably reduced in about 70% of the patients, and this parameter is closely associated with clinical disease activity.

The many different kinds of more or less well-defined conditions and states with acquired CMID are insufficiently explored, but development is under way. To understand the pathogenesis, we can so far only make assumptions about the mechanisms. The T-lymphocytes consist of several functionally defined subpopulations, and defects of one or more of these populations can combine in different pathogenic patterns. If the CMID affects mainly the T-effector lymphocytes, the cell-mediated inflammatory process will be quantitatively and qualitatively deficient. This deficiency may have very serious consequences, since the T-effector lymphocyte is essential for the combat of infection. A well-known example is infection-associated CMID in lepromatous lepra (6). After infection the elimination of the lepra bacterium and other mycobacteria is dependent upon an adequate cell-mediated immune reaction, and in this respect circulating antibodies have no importance. A person with a normal T-lymphocyte system develops tuberculoid lepra with lesions containing only a few bacteria, and this type of lepra infection generally takes a benign course, the bacteria being checked by the T-lymphocyte system. In lepromatous lepra, however, this specific checking mechanism is out of order, the bacteria proliferate, the lesions are swarming with living bacteria, the clinical course is quite different, and the disease is more contagious.

It is probable, that the T-effector lymphocyte and its functions are also of importance for the combat of a certain malignant tumours, although this is fervently discussed by experts (14). The T-effector lymphocyte is responsible for the cutaneous contact eczema, which is used in assays that evaluate the function of the T-lymphocyte system by sensitization of the skin. T-lymphocytes are also important in the inflammatory tissue damage associated with allografting and with certain autoimmune diseases, although this indeed does not mean, that persons with CMID avoid autoimmune diseases; on the contrary, they are affected more often than normals, perhaps because they lack T-suppressor cell activity (1, 14).

Lack of T-helper lymphocyte function implies deficient help to B-lymphocytes to produce antibodies, which may sometimes make a CMID case resemble a case of combined immunodeficiency, since there may be a quantitative and/or qualitative lack of circulating antibody. However, the true mechanism is revealed if normal B-lymphocyte function and antibody production is restored by successful treatment of the T-lymphocyte defect. Defect of the T-suppressor lymphocyte function leads to deficient regulation of the immune system. As is the case with most information on the function of T-lymphocyte subclasses, knowledge on the T-suppressor lymphocytes (9) is mainly derived from animal experiments, but techniques for measuring T-suppressor function in man are being developed and clinically tried for the moment. As the name indicates,

the T-suppressor lymphocytes can suppress and thereby regulate both T-lymphocyte activity and B-lymphocyte activity with associated antibody formation. Lack of this regulating mechanism may for instance cause an exaggerated B-lymphocyte response, which is actually seen in some immune deficiency states as for instance congenital thymic aplasia. If the T-memory lymphocyte function is deficient, this becomes manifest through insufficient or absent immunological memory to previously experienced antigen. The defect may be quantitative as well as qualitative: Broadly comprising all antigens or selective for only a single or a few antigens.

SYMPTOMS AND SIGNS

In spite of all our knowledge of T-lymphocyte subclasses and their function and non-function, we have so far limited exact knowledge of relevance for the clinic. But there is good reason to believe, that the whole field will continue to develop and expand in the near future.

As to clinical signs and symptoms, these are obvious and well-known, but perhaps not always sufficiently well recognized and diagnosed. One major group of symptoms are those due to infection, prevalently infection with such bacteria, that live and proliferate within the cells, and infection with viruses. Typical infectious agents are candida, other fungi, pneumocystis carinii, cytomegalo virus, herpes virus and other microorganisms, that are seldom causing infection in persons with a normal immune system (10, 17). There is a deficient inflammatory response in lymphocyte mediated immune inflammation, the lymph nodes show characteristic changes with T-lymphocyte depleted paracortical areas, demonstrable by adequate biopsy. In the congenital forms of CMID, there are often obvious signs of pharyngeal pouch malformation and such signs, e.g. hypofunction of the parathyroid may be overlooked and thus remain temporarily untreated. In all the acquired forms of CMID, the symptoms of the primary cause may dominate the clinical picture and also the thoughts, associations and diagnostic work of the clinician. An evaluation of the immune system should actually be included in any extensive clinical examination of a patient in quite the same way as is the case with parameters for estimation of liver function, kidney function, hemopoiesis etc. If a primary diagnosis can be made and the primary disease cured, the secondary CMID will usually disappear, but in the choice of therapy and management of the patient, it may often be highly recommendable to monitor also the immune function, i.e. by assays, which register T-lymphocyte functions and cell-mediated immunity. Such assays are being used and developed in specialized laboratories and departments today and shall eventually be available at a larger scale to the clinic. Some of these

assays are mentioned in Table 1: one very easy thing to do is to count the lymphocytes of the peripheral blood. The conventional total count of white blood cells with the lymphocyte number given as a percentage of the total count is not optimal, and absolute values of the true blood concentration of lymphocytes and of each individual cell type is preferable. Technically it is not difficult, but somewhat laborious to count the number of T-lymphocytes and B-lymphocytes in peripheral blood. Functional lymphocyte tests are becoming more and more used. If lymphocyte containing material can be obtained from other sources than blood, such as joint fluid or pleural exudate, quantitative and qualitative examinations of lymphocytes can be performed. The lymphocyte transformation response to bacterial lipopolysaccharide, phytohemagglutinin, concanavalin-A, antigen and allogeneic lymphocytes is important for evaluation of the T-lymphocyte dependent immune system. The capacity of lymphocytes to produce lymphokines in response to non-specific and antigen-specific stimulations has been used in some studies and given interesting and promising results (2). In specialized departments an even larger number of assays are developed and used to diagnose and monitor CMID (2, 3, 7, 8, 13, 17).

MONITORING AND THERAPY

The experience derived from the exploration, classification, diagnosis and treatment of inborn immune deficiencies has been a great initiating impulse, and all the experience and technical developments, that has led up to new assays and methods of examination, can now be used to diagnose and monitor the acquired immune deficiencies, also those that are induced by immunosuppressive or cytostatic therapy, in which we are still without a clearly defined functional picture of the drug induced modulation of the immune system. Many treatments interfere seriously with the immune function, and the clinician does not possess sufficient possibility to monitor his therapy; but future increased use of adequate techniques might enhance progress in this field. At present, immunosuppressive and cytostatic treatment in several medical disorders is conducted on an empiric basis without sufficient monitoring, and a similar lack of prospective monitoring would indeed not be accepted in treatment of other organ systems, e.g. heart, lungs, liver, kidneys or gastrointestinal canal. Fortunately enough this is becoming increasingly recognized, clinical immunology is developing adequate methods, and techniques for immunological monitoring are becoming more available and more used.

The main points in the treatment of CMID are to treat infection and to prevent infection, since infection is always the dominating symptom. If the CMID is acquired, the primary disease must be treated, thereby correcting the cause

of the immunodeficiency. If the CMID belongs to the congenital type, the treatment is substitution of the deficient or lacking cell population by transplantation (5). In all cases more or less extensive monitoring before, during and after treatment is necessary, and team-work of large groups of specialists is needed.

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Therapy of immunological deficiencies

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Appropriate treatment depends on accurate diagnosis, and can be difficult with the secondary deficiencies (see Hobbs, 1974), e.g. the catabolism of IgG can be increased ninefold. Today, available treatments can be summarised under the following headings: (i) forewarned is forearmed, (ii) immune globulins, (iii) plasma, (iv) immunostimulants, (v) leucocytes, (vi) transplantation.

i. Forewarned is forearmed

Until T-lymphocyte deficiency is excluded, any transfusions should be irradiated with 1,500 Rad to prevent graft-versus-host disease. Forewarning enables the patient's doctor immediately to institute appropriate therapy at the onset of any potentially infectious disease. On the whole, prophylactic antibiotics are to be avoided except where resistant strains are very rare in occurrence, e.g. streptococcal susceptibility or pneumocystis carinii. For deficiencies of intracellular killing by phagocytes, antibiotics (Chloramphenicol, Rifampicin) gaining entry to the leucocytes are preferred. Hyperimmune globulin (see ii), fresh plasma (iii) or leucocytes (v) can be used early. In IgM deficiency (see Hobbs 1975), incipient fatal septicaemia can be averted with IgM anti-endotoxin (Kim and Watson, 1965) or normal fresh plasma.

For some selective immune deficiencies, e.g. only in phagocytosis, a fuller programme of prophylactic immunisation can be used to encourage compensation by the other limbs in immunity. In general, however, live attenuated vaccines (oral poliomyelitis, cow-pox, measles, BCG, etc.) must not be used where there is severe T-cell or IgM deficiency, for fatal disseminated disease has resulted in such patients. It is wise to screen for the commoner complications such as tuberculosis (by chest X-ray), leucopaenia or haemolytic anaemia (by blood counts and spleen size), malabsorption (by serum albumin) indolent inflammation (by ESR, C3 level) autoantibodies (by fluorescence) or neoplasia (especially where there are chromosomal abnormalities as in ataxia telangiectasia).

ii. Immune globulins

This must be considered obligatory in children with hypogammaglobulinaemia, or 70% will die within two years if it is not given (MRC 1969). Where

reactions occur (20% of patients) not due to antibodies to IgA (Schmidt *et al.*, 1969), plasma exchange therapy is better, veins permitting.

Hyperimmune gammaglobulins are finding wider use today (see Table 1).

TABLE 1

Use of gamma globulins (see also Janeway and Rosen, 1966)

1. <i>General prophylaxis</i> (reactions to aggregates, IgA!)			
Try 50 mg/kg/2 weeks			
or 50 mg/kg/week			
2. <i>Following exposure</i>		<i>Ambient γ-globulin</i>	
Measles, hepatitis	25 mg/kg	<i>Hyperimmune γ-globulin</i>	
Poliomyelitis	50 mg/kg	Mumps, tetanus	50 mg/kg
Rubella, herpes	100 mg/kg	Vaccinia, pertussis, varicella	100 mg/kg
		Pneumococcal	
		Staphylococcal	
		Diphtheria	

iii. *Plasma*

The plasma of choice is that freshly collected from volunteer donors already screened for transmissible diseases such as hepatitis and cytomegalovirus. Where its collection can be contaminated with living cells, then it is important to freeze and thaw, but this does increase the incidence of urticarial and other types of reaction which will eventually occur in about 25% of all recipients.

While these can be covered by prior administration of antihistamines and, where indicated, intravenous adrenalin and hydrocortisone, the use of more modern methods with high velocity centrifugation can remove living cells from the plasma, which then does not need to be denatured in any way. A doctor with an airway should always be present for the first 30 minutes, as anaphylaxis mainly kills when no one is prepared for it. PPF (plasma protein fraction) is not suitable as a source of either gamma globulin or useful complement.

Plasma therapy can either be given as 250 ml/weekly or by cell separator as a 2-litre exchange every three weeks (Oon and Hobbs, 1975). A small panel of known donors can be much safer than the use of a transfusion service with regard to transferred hepatitis. In hypogammaglobulinaemia with malabsorption, we have witnessed weight gains of over 20 kg from plasma after gammaglobulin has failed, presumably because the plasma also contained useful IgA and IgM.

Hyperimmune plasma can be especially valuable for infections with *Ps. pyocyaneus*, *K. aerogenes*, *E. coli*, *H. influenzae*, *N. meningococcus*, measles, Varicella, Herpes simplex, *Staph. aureus*, *Pneumococcus* etc. (Hobbs, 1977).

For certain deficiencies of the complement system, it is possible to test the effect of the addition of normal fresh plasma and, where such defects are cor-

rected by low concentrations (10-40%), fresh plasma therapy should be considered, initially trying 250 ml/weekly or covering an infectious episode or risk. Some patients can make antibodies which inactivate the replacement components, so it is worth checking the activity of the post-transfusion plasma. In this way, an error on the alternate pathway of complement was corrected in twins who had had osteomyelitis at several diaphyses for over 8 years and healing occurred within one year (Hobbs, 1977).

iv. *Immunostimulants*

Personal experience of the use of immunostimulants in now a very large number of patients suggests that those who have derived most benefit have been the patients in whom it has been possible to demonstrate some reactivity, chiefly of their T-cells, but at a sub-normal level: i.e. where there is already some capacity to respond, immunostimulants can elevate responses into the normal range. Occasionally, (imbalance between T-suppressor and T-helper functions?) there can be an overshoot of the B-cell responses. On the other hand, in about half of our patients who never seem to have antibodies to their malignant melanoma, we have never succeeded in generating them with combinations of autologous irradiated tumour cells and immunostimulants. It is the other half of patients with pre-existing antibodies who have shown the positive responses. Current immunostimulants include transfer factor, Levamisole and Thymosins.

All three of these have produced responses in the granulomatous form of chronic mucocutaneous candidiasis (Type 2, Valdimarsson *et al.*, 1973). It also appears that transfer factor prepared from a donor hyperimmune to the response to be stimulated can achieve effects lasting 4-8 weeks longer than those from ordinary donors. Thus there is some specificity for transfer factor, that is not the case for Levamisole and Thymosin. Transfer factor has not been impressive in Wiskott-Aldrich syndrome and has failed in ataxia telangiectasia. The use of Levamisole (Willoughby and Wood, 1977) and Thymosins (Goldstein *et al.*, 1977) have been well reviewed elsewhere.

v. *Leucocytes*

The advent of cell separators and filter-collection of phagocytes (with protection against autoactivation by high dose of ascorbic acid) has greatly improved the possibility of treatments and recent developments have been well reviewed (Heim, 1977).

One new approach, however, that seems worth mentioning here is the use of competent leucocytes sharing a haplotype with the recipient, to programme the host's lymphocytes where their defects could be secondary to a failure of the

correct phagocyte-lymphocyte interaction. Present concepts are summarised in Fig. 1, where we believe the receptors for specific response to immunogens reside on the T- and B-cells but they, together with the phagocytes, carry other surface components that are essential for them to interact with each other, illus-

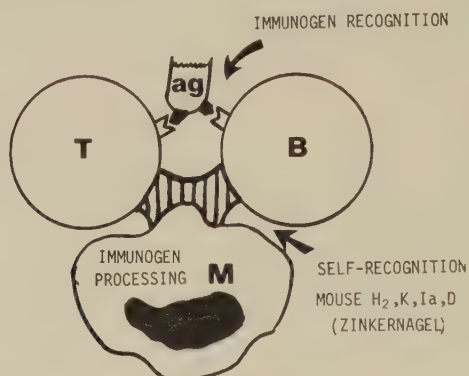


FIG. 1. The dual recognition concept; specific recognition of immunogen is a function of receptors on T- and B-lymphocytes, but the correct interaction between these and macrophages also require self-recognition with a complete match in the mouse on the H₂, K, Ia and D alleles.

trated as selfrecognition. In elegant reconstitutions of irradiated inbred mice, Zinkernagel has shown that for the correct interactions the match must occur on the H₂, K, Ia and D loci. Table 2 perhaps shows how this applies in the human situation. In 1974 we recognised 2 children who had been afflicted with recurrent infections for many years as having a lazy leucocyte syndrome, but

TABLE 2

Effect of irradiated (1500 R) one haplotype-identical normal WBC on functional T-cell deficiency secondary to lazy leucocytes

	Increment in CPM			
	PHA	Candida	PPD	MLR
April 74	600	20	—15	1,050
Pretherapy				
Sept. 74	1,030	30	+30	1,850
Therapy 4.3×10^6 Donor WBC				
8 days	3,500	2,600	2,400	11,200
Post-therapy				
4 yrs	—	14,800	—	36,300

who also showed very poor function in the T-lymphocytes. One had received fresh blood transfusion without any benefit. I considered their T-lymphocyte deficiency might be secondary to inability of the incompetent phagocytes to programme: and so used the parental leucocytes (after 1,500 Rads) to see what would happen. Within 8 days healthy responses appeared against all the immunogens tested, and these have been maintained throughout the past four years, during which time both children have not required a single admission to hospital and are leading normal lives.

These new concepts of haplotype co-operation being essential between lymphocytes and phagocytes have led us to think again about the selection of bone marrow donors (see Table 3).

TABLE 3
Bone marrow donors

1. Identical twin	4. Haplotype-identical (unrelated)
2. Sibling	(i) chance compatibility
3. Other family	(ii) fractionated
(i) chance compatibility	(iii) ATG-treated
(ii) induction of specific unresponsiveness	(iv) Cyclosporin-A

With the ideal donor as an identical twin, the next best is a sibling sharing identical haplotypes. Sometimes by tissue-typing the whole family a further member can be found who shares one or both haplotypes and is histocompatible and in 1971 (Hobbs *et al.*, 1976), we performed a successful graft from a father to a son who was afflicted with a severe combined immune deficiency disease, since when similar grafts have been successfully obtained elsewhere from an uncle, an aunt and even a great-uncle. Two years ago we were told at Westminster that our panel of HLA-typed unrelated donors would shortly become obsolete with the advance of D-typing, but I have yet to hear of a successful graft based solely on D-typing, and the above concept indicates that donors should be haplotype-identical for the other loci as well. Apart from transient successes, grafts from unrelated donors have more often ended in graft-versus-host disease than in long-lasting cures, of which only two are recorded. The idea that fractionation of the bone marrow could remove competent lymphocytes and allow the slow emergence of new ones tolerant to the new host (Dicke *et al.*, 1975) has not been supported, and indeed this procedure to date has usually delayed the onset of eventually fatal graft-versus-host disease. Another new approach has been to strip the bone marrow graft of competent lymphocytes with a well-absorbed anti-thymocyte globulin. Using inbred rodents, it is known

that if an irradiated F1 hybrid receives a bone marrow graft from a parent, this usually results in fatal graft-versus-host disease in 100%. With prior ATG treatment of the bone marrow, successful grafts have been obtained experimentally, and it remains to be seen whether this will work in the human situation. A new drug, Cyclosporin A, is currently in very short supply but appears to have a unique action in that it is toxic to the co-operation between the T-lymphocyte and the phagocyte, but non-toxic to bone marrow stem cells. Its use prior to inducing a primary immune response not only prevents that response but abrogates it so that it cannot again be induced although the drug is no longer used (Borel *et al.*, 1977). On the other hand, were a secondary immune response is already established, this reappears as soon as the drug is stopped. This drug is therefore being explored for bone marrow transplantation. The last possibility is the induction of specific unresponsiveness which has been beautifully achieved in rats by Binz and Wigzell (1978). The principle is illustrated in Fig. 2.

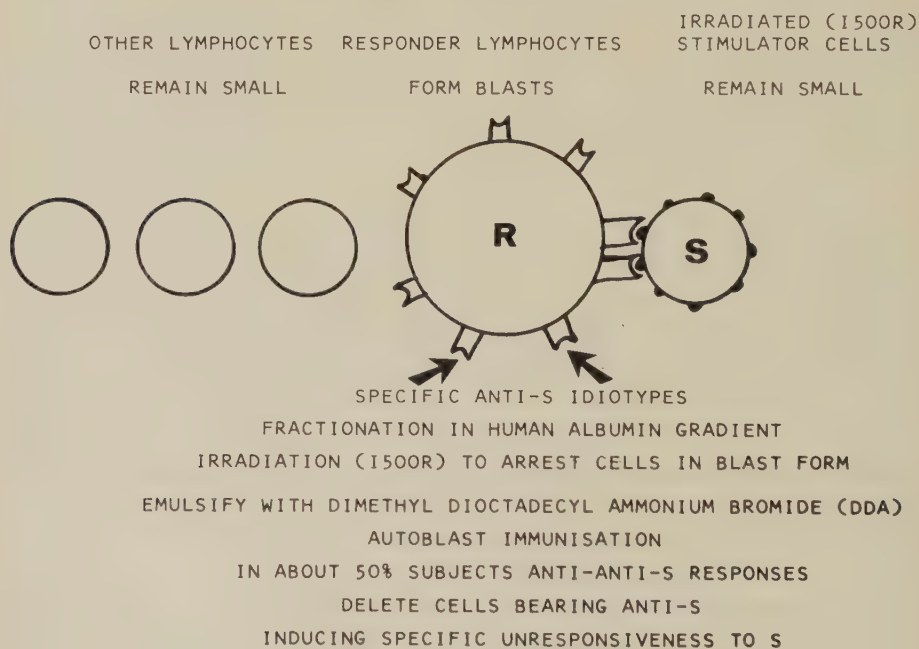


FIG. 2. Induction of specific unresponsiveness.

To apply this in the human situation, we have chosen to irradiate the stimulator cells so that no poisonous drugs will be given to volunteers, and the fractionation of the culture on the fifth day is on a human albumin (non-antigenic) gradient. It has been possible to obtain a preparation of blasts 98-99% pure.

We have then irradiated these at 1,500 Rad to arrest them in the blast stage when they are bearing an excess of the specific idotype recognising the stimulator cells. Freund's complete adjuvant has had some serious reactions in humans, though we have tried a new adjuvant DDA which has been shown by Snippe *et al.* (1977) to be about twice as effective as Freund's and in five humans to date we have not observed any serious side effects following extensive intra-dermal immunisation. Table 4 shows the results we recently achieved for a mother

TABLE 4
The induction of specific unresponsiveness

	Increment in CPM	
Responder cells	Mother	Mother
Stimulator cells	son	pool of 5
Before	23,000	45,000
<i>Autoblast immunisation</i>		
After	400	43,000

against her son, reducing her specific responsiveness to a background level of 400 CPM, yet not affecting any of her other immune responses, nor inducing any autoantibodies at 1 and 4 months. The tragedy was that her son died the day before he was to receive his mother's bone marrow. For three further prospective family donors, we did not achieve abolition of the specific MLR response, but this month have again achieved it for a father against his son, who has severe combined immune deficiency. The bone marrow graft is being done this week and will put this approach to the test, for if it is successful it opens a practical procedure for patients without compatible donors but where the shared haplotype can be guaranteed.

vi. *Transplantation*

Around the world, foetal liver and/or thymus of out-bred origin has been used over 50 times and for all those cases reported at least one year later death has ensued, probably from fatal graft-versus-host disease, and reconstitution has been wanting. In view of the requirement for a completely matching haplotype, I believe this approach is now obsolete. For less serious life threatening deficiencies, an approach introduced by Valdimarsson *et al.* (1972) was the infusion of competent leucocytes from a healthy to a diseased sibling. I can now report that this corrected the lymphocyte deficiency of the recipient, completely for five years, but with slowly decreasing efficiency over the next two years,

until seven years later the boy relapsed with chronic mucocutaneous candidiasis. Throughout this time there was no rejection of his brother's lymphocytes, and so we were able to repeat the process again with immediate correction, and are just waiting to see how long this will last. The advantage of this procedure is that it avoids all the trauma of a formal bone marrow graft.

Bone marrow grafts for immunodeficiency are really of two major types; the first to replace clones absent in the patient (e.g. absent stem cells in a lymphopaenic SCID) or to displace useless clones which may be occupying a large amount of the marrow space. In the former situation over 80 patients have been treated with a success rate of about 65% simply following infusion of the bone marrow. In the latter situation there is less experience but success can be achieved (Foroozanfar *et al.*, 1977). Our team have introduced a method whereby the pelvis of a child can be totally irradiated without damaging other vital organs, and have seen a selective graft settle in such a pelvis. This is considered necessary where there is hyperplasia of useless clones, e.g. the myeloid excess usual in chronic granulomatous disease, which can almost mechanically crowd out an attempted graft.

The initial optimism (Polmar *et al.*, 1976) that red cell therapy could correct adenosine deaminase deficiency (ADD) and SCID has not been borne out in at least 8 subsequent cases, mostly diagnosed after 3 months of age when it appears that secondary changes such as thymic atrophy and exhaustion of the maternal reserves result in non-response. To be a real success, it seems this therapy needs to be instituted within a few weeks of birth.

Finally, I would like to report our most recent graft to severe combined immune deficiency where the donor was unrelated and it seems graft-versus-host disease has been abrogated by the use of Cyclosporin A. The infant was not diagnosed until aged 5 months and was ADA-deficient with total lack of responsiveness to red cells together with Thymosin. Fig. 3 summarises the evidence for total absence of B- and T-lymphocyte function. From the 20,000 volunteer panel kept by my colleague, Dr. D.C.O. James, an ABO-haplotype identical unrelated donor was found who appeared histocompatible with the infant. Despite such a match on a previous occasion, we had experienced a fatal graft-versus-host result. We therefore decided to cover this graft with Cyclosporin A starting on Day -2 and my colleague, Professor J.G. Humble, collected the bone marrow, using the closed technique he himself introduced in 1956. The Cyclosporin A produced further lymphopaenia and indeed the diarrhoea worsened so that it was stopped at Day 8. Seven days later, with the rising lymphocytes of a graft, acute graft-versus-host disease appeared with a widespread rash, fever and an aspartate transaminase level of 200 I.U. That same day the drug was reintroduced, together with additional methotrexate, which had been used

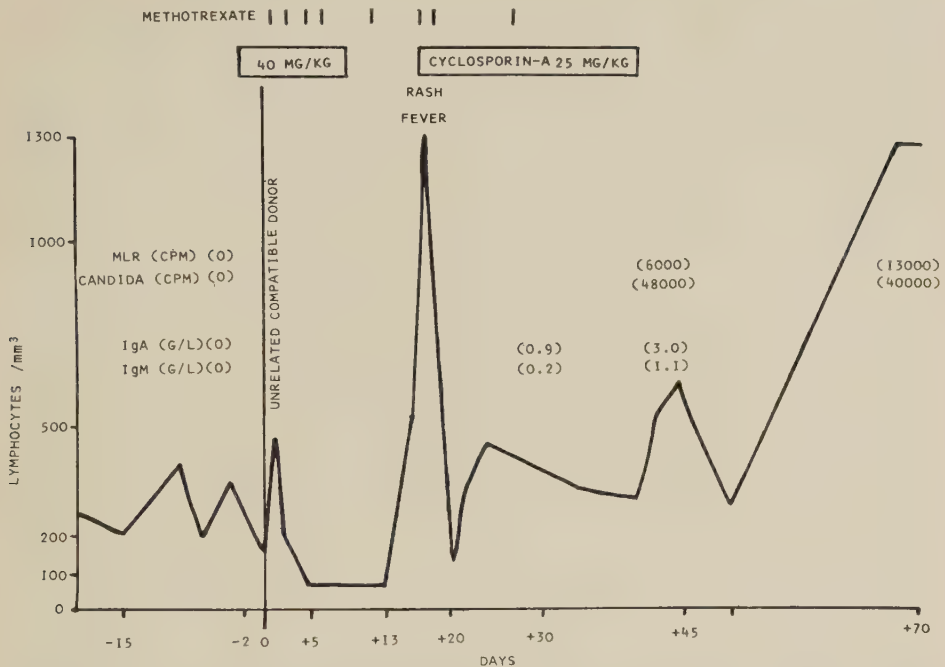


FIG. 3. Use of methotrexate and Cyclosporin A to abrogate graft-versus-host disease following an unrelated graft to an infant with SCID.

in the Seattle manner but had not prevented this reaction. Again lymphopaenia ensued, and the reaction disappeared while the full 30 das was completed as recommended by Borel *et al.* On stopping the drug, a fully competent graft emerged and the enormous candida responses were associated with complete clearing of the mucocutaneous candidiasis this infant had had since birth. At 75 days there has been no further evidence of graft-versus-host disease but perhaps it is too early with this first human success to make any further speculations. It seems worth adding that this is only the third unrelated donor from whom the Westminster team have achieved a successful graft without a fatal graft-versus-host reaction and the donor did share the necessary haplotypes to achieve reconstitution.

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SYMPOSIA
AUTOIMMUNITY
AND AUTOIMMUNE DISEASES

Progress in the diagnosis of autoimmune diseases

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INTRODUCTION

In principle the diagnostic tools for autoimmune serology are not different from those used in the fifties, when it became clear that autologous antigens can be specifically coated by the patient's own antibodies (e.g. positive direct anti-globulin test on red cells) or that sera from patients contain antibodies against the patient's own tissue substrates (e.g. antibodies to thyroglobulin). All serological methods use either antibodies against human immunoglobulins to demonstrate the presence of autologous antibodies on their target cells or they offer autologous antigens to the serum of the patient, taking care that by a subsequent trick the presence of these antibodies on the auto-antigen can be demonstrated. It should be kept in mind that auto-antibodies are not at all individual-specific and mostly not even species-specific. For the demonstration of these auto- and iso- and hetero-antibodies therefore often antigens of animal-origin can be used. However, in many of the recent methods, which will be discussed in this paper, the use of fresh substrates of *human* origin is of importance.

Concerning the techniques for autoimmune serology the immunofluorescence technique (IFT) still leads the field, followed by agglutination and precipitation methods. Enzyme-linked-specific-antibody (ELISA) methods may come to replace radioimmuno-assays (RIA).

In Table 1 a long list is given of diseases, which are considered to-day as autoimmune diseases or at least diseases with autoimmune aspects. In some cases the arguments in favour of autoimmunity are extremely weak (i.e. ulcerative colitis, vitiligo) and mainly of historical (i.e. dermatomyositis) or futuristical (i.e. lymphocytic aplasia) nature. The diseases mentioned in Table 1 are divided in five groups. Only the division between group I and II+III+IV+V is of principal importance since it divides diseases of which we know, or at least suspect, a certain etiology from others which we still have to classify as idiopathic. Classification among group I does not at all include a known pathogenesis.

The antibodies mentioned in Table 1 are just those which are generally considered helping to differentiate the mentioned disease from other diseases. It should be underlined, however, that many of these auto-antibodies also occur in other autoimmune diseases, sometimes even in high frequencies. Most of these antibodies are known already for a long time and will therefore not be discussed in this paper. Some of the recently discovered antibodies will probably be extensively dealt with by the other speakers at this symposium.

RECENT DEVELOPMENTS

Dr. Schoysmann will certainly mention the auto-antibodies to sperm which appear in 70% of vasectomized men. He probably will also speak about those to the zona-pellucida of egg-cells and possibly also about the auto-antibodies to ovarian and testicular steroid-hormone producing cells.

I expect that Dr. Bastenie will discuss extensively the clinical value of the antibodies to islets of Langerhans. Probably he will also spend a few words on thyroid autoimmunity, it being his old favorite. I therefore only shortly mention the progress which is made for the diagnosis of *Graves' disease* by the introduction of a non-bio assay for the demonstration of antibodies to thyroid membrane receptors for TSH. These antibodies, previously indicated as long acting thyroid stimulator (LATS), if directed to mouse-thyroid, or LATS-protector if directed to human thyroid, are now mostly indicated as *thyroid stimulating antibodies* (TSAb). They can be demonstrated with a radio-immuno competition assay according to Smith and Hall (1974). The principle is given in Figure 1.

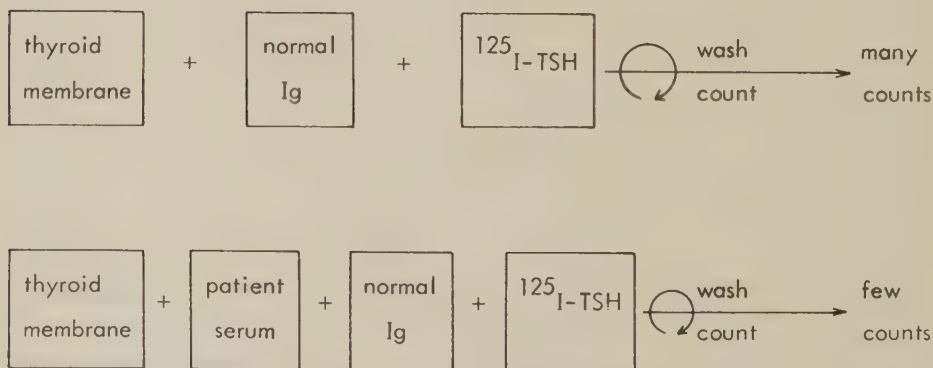


FIG. 1. Demonstration of thyroid stimulating antibodies (TSAb) according to Smith and Hall, 1974.

TABLE I

Autoimmune diseases and their antibodies

Disease	Antibody	Disease	Antibody
I. Secondary autoimmune diseases			
Altered auto-antigens		Autoimmune steroid-hormone deficiency	adrenocortex
Postmyocardial infarction syndrome	heart	Addison's disease	steroid hormone producing cells
Post-pericardiotomia syndrome	heart	Primary ovary deficiency	adrenocortex and others
Haemolytic anaemia with ovary tumor	red cells		
Sympathic ophthalmia	uvea	Vitiligo	parathyroid
Goodpasture's syndrome	basement membranes (GMB, TBM)	Autoimmune parathyroiditis	islets of Langerhans
Active chronic hepatitis	smooth muscle; LMA	Idiopathic hypoparathyroidism	
	LSP	Autoimmune insulitis	
Pemphigus	epidermal substance	Juvenile diabetes mellitus	
Pemphigoid	dermal basement membrane	III. Other idiopathic autoimmune diseases	
Coeliac disease	bran	Primary biliary cirrhosis	mitochondria
Drug-induced autoimmunity	wheat, reticulin (complicated)	Myasthenia gravis	acetylcholine receptors
Exogenous antigen mimicing auto-antigens		Thymoma	skeletal muscle
Rheumatic carditis	heart	IV. Connective tissue diseases	
Ulcerative colitis	colon	Systemic lupus erythematosus	nuclei (ANA = ANF); dsDNA
Encephalomyelitis following rabies vaccination	brain	Rheumatoid arthritis (RA)	IgG (RF), perinuclear Ag (APF)
Pemphigus Brasiliensis	epidermal basement membrane	Sjögren's syndrome	salivary duct cells
Leakage of sequestered antigens	lens	Scleroderma	nuclei
Facogenic uveitis	sperm	Dermatomyositis	
Vasectomized men		Mixed connective tissue disease (MCTD)	extractable nuclear Ag (ENA)
II. Organ specific autoimmune diseases			
Autoimmune thyroiditis		Chronic discoid lupus erythematosus (CDLE)	
Hashimoto's disease	thyroglobulin, cytoplasm	V. Autoimmune diseases of blood and bone marrow	
Primary myxoedema	thyroglobulin, cytoplasm	Autoimmune haemolytic anaemia	red cells
Graves' disease	thyroid stimulating Ab	Pure red cell aplasia	erythroblasts
Autoimmune gastritis	parietal cells	Autoimmune thrombocytopenia	platelets
Atrophic gastritis	parietal cells, intrinsic factor	Megakaryocytic aplasia	megakaryocytes
Pernicious anaemia	parietal cells, intrinsic factor	Autoimmune granulocytopenia	granulocytes
Combined cord disease		Granulocytic aplasia	myelocytes
		Autoimmune T- or B-lymphocytopenia?	lymphocytes
		Lymphocytic aplasia?	

Dr. Serafini will tell you about all recent developments in gastric autoimmunity and Dr. Meyer zum Büschenfelde will probably discuss the latest views on the important antibodies to liver membrane antigens (LMA). I wonder whether he will agree that I listed active chronic (HBsAg negative) hepatitis among the secondary autoimmune diseases (Table 1).

I will in the rest of my paper only discuss the recent progress made on the demonstration of antibodies to wheat, to acetylcholine receptors, to dsDNA, to IgG, to perinuclear antigens, to granylocytes and to platelets.

Antibodies to wheat

In gluten sensitive patients oral intake of wheat proteins gives rise to antibodies against gluten and gliadin. Eterman *et al.* (1978) recently developed a simple method to demonstrate these antibodies. Cryostate sections of wheat grains were used as a substrate for the indirect IFT. The antibodies to wheat, demonstrated in this way are sensitive markers for gastro-intestinal diseases, but they are not specific for gluten enteropathy. Antibodies to reticulin are less sensitive, but of greater specificity for coeliac disease (Table 2).

TABLE 2

Frequency of antibodies to wheat and reticulin

	<i>No. studied</i>	<i>Ab to wheat</i>	<i>Ab to reticulin</i>
Untreated coeliacs (adults)	36	50%	33%
Treated coeliacs (adults)	59	32%	10%
Crohn's disease	50	52%	6%
Ulcerative colitis	44	18%	7%
Controls (adults)	95	4%	2%
Untreated coeliacs (children)	6	100%	33%
Treated coeliacs (children)	8	87%	0%
Controls (children)	60	6%	0%

Antibodies to acetylcholine receptors

Such antibodies are probably directly responsible for the pathogenesis of myasthenia gravis. However, the radio-immuno precipitation method, which mostly is used to demonstrate these antibodies is complicated (Lindström, 1977). Sondag-TSchroots *et al.* (1978b) recently showed that it is also possible to use

the simpler indirect IFT ith rat diaphragm as a substrate, if the location of the antibodies on the motor-endplates is confirmed by using a-bungarotoxin. A double staining method as described in Figure 2 is needed. The method is specific but not sensitive (Table 3). The latter may be overcome by the use of fresh human muscle tissue as a substrate.

4th incubation	TRITC-labelled anti- α -bungarotoxin
3rd incubation	α -bungarotoxin
2nd incubation	FITC-labelled anti-human Ig
1st incubation	Serum patient
Substrate	Rat diaphragm

FIG. 2. Incubation scheme for the demonstration of antibodies to motor-endplates.

TABLE 3

Frequency of antibodies to skeletal muscle and motor-endplates

	No. studied	Ab to muscle	Ab to motor-endplates	Total
Myasthenia gravis	57	26%	21%	47%
Other neuromuscular diseases	22	0%	0%	0%
Normal subjects	50	0%	0%	0%

Antibodies to double stranded DNA (dsDNA)

These antibodies are very specific for systemic lupus erythematosus and peaks in the level of these antibodies often preceed exacerbations of the disease (Swaak *et al.*, in preparation). The methods used by most investigators are the Farr assay and the IFT (Aarden, 1977a). The latter method is simple and only slightly less sensitive than the Farr assay. Haemoflagellates (e.g. *Crithidia luciliae*) are used as a substrate (Aarden *et al.*, 1975). They possess a giant mitochondrion with a kinetoplast consisting of a network of rings of dsDNA without histones. The Farr assay only detects high avidity antibodies, which can stand a high ionic strength. These antibodies are bivalently bound to one dsDNA molecule. If these antibodies are of the IgG class small complexes are formed. Such antibodies often occur in sera from SLE patients with renal involvement (Aarden, 1977b).

Antibodies to perinuclear antigens (APF)

These antibodies mostly indicated as anti-perinuclear factor (APF) are not at all new, since they were described already in 1964 by Nienhuis and Mandema. Although these authors reported a good specificity for rheumatoid arthritis (RA) of these antibodies, their results, which only were confirmed in Italy and France, never threatened the monopoly of the rheumatoid factor as sero-diagnostic tool for RA. A recent re-evaluation (Sondag-TSchroots *et al.*, 1978a) of the APF test showed, however, that the APF test is not at all less sensitive or less specific than the classical rheumatoid factor test (Table 4).

TABLE 4

Frequency of APF and IgG-RF in comparison with classical RF tests

	<i>No. studied</i>	<i>Rose test</i>	<i>Latex test</i>	<i>IgG-RF</i>	<i>APF</i>
RA	143	57%	62%	66%	70%
Degen. Joint Dis.	32	3%	3%	0%	6%
Ankyl. Spondylitis	50	2%	13%	4%	4%
SLE	73	5%	8%	33%	10%
Healthy subjects	161	1%	2%	0%	2%

IgG rheumatoid factors (RF)

By various investigators methods have been described for the determination of RF of the IgG, IgA as well as the classical IgM class (Torrigiani and Roitt, 1967; Estes *et al.*, 1973; Hay *et al.*, 1975). This might be of importance since IgG/anti-IgG complexes are considered to form the nucleus of complexes locally produced in the joint of RA patients which after stabilization and enlargement by IgM RF lead to a continuous phagocytosis with leakage of lysosomal enzymes. The demonstration of IgG RF is also of diagnostic importance since, as also Dr. Verweij-Burke from our department showed, the number of sero-negative RA patients shows a considerable decrease with retention of specificity except for SLE patients, of which 33% have IgG RF if demonstrated with an IFT (Table 4). Even more SLE patients have non-agglutinating IgM RF.

Antibodies to granulocytes

For years many workers tried to find satisfactory methods to demonstrate these antibodies. Leuco-agglutination tests could be used for the demonstration of iso-antibodies but not for the study of auto-antibodies. Also a satisfactory equivalent of the anti-globulin test was not available until it was shown by Verheugt *et al.* (1977) that granulocytes which were fixed in paraformaldehyde 1%

could be used as a substrate in the indirect IFT. With this fixation for the first time proper negative controls were obtained. Using Fab or F(ab')₂ FITC labelled anti-Ig reagents also the last remains of nonspecific membrane fluorescence through binding via Fc receptors vanished.

Antibodies to platelets

The same fixation, which proved to be successful for the determination of auto-antibodies to granulocytes could also be used for the demonstration of auto-antibodies to platelets in sera from patients with idiopathic thrombocytopenia (ITP). In 70% of ITP patients such antibodies were found. Most of these antibodies were of the IgG class.

The above method also allows the detection of antibodies already bound to the patient's own platelets. An equivalent of the direct anti-globulin test on red cells is therefore now available.

CONCLUSION

In summary we may say that although in principle the methods for the study of autoimmune serology have not altered, a steady progress in filling the white spots in the map of autoimmune diseases could be noted. The progress in the field of anti-acetylcholine receptor and anti-TSH receptor serology might stimulate further research for other anti-receptor antibodies interfering with hormone or cell-cell interactions.

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Immunological infertility

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Immunological infertility is one of the controversial subject in the field of the couple sterility. Since several decades it is known that there exist different types of antibodies which in the and make it impossible for the spermatozoa to penetrate into the cervical glare or into the corona radiata of the egg. The fact that the spermatic cell possesses several subdivisions made it possible to describe antibodies against the protein substances of different cell levels. But in the numerous substances whose existence has been recognized, there can be classified three large types of antibodies, i.e. agglutinins, immobilizines and cytotoxic antibodies. Since the agglutinins are the less difficult finding substances, their role in couple infertility is the one that is mostly dealt with in literature. There are however pathological situations in which only the one or the other of these types of antibodies exist. According to the rates present in the blood of the one or the other of the two partners of the couple, one is faced either with subclinical situations or with clinically recognized situations. Among the clinically recognized situations there is the autoagglutination of the spermatozoa in the ejaculatio studied of a man with diminished fertility, and there is on the other hand the negativeness of the invasion tests since the cervical glare is put into contact with spermatozoa.

These two situations are easily put in evidence under the microscope and direct to the diagnosis of couple infertility towards the immunological infertility.

Neither recognizes a diagnostic value in the presence of agglutinin antibodies, be it in the man or in the women, even if the clinical objectivation criteria are lacking. The etiology of this situation in one of the two partners of the infertile couple remains in most cases unknown. In a small number of cases of masculine autoagglutination, there may be found lesions of the epididymis which lead to think of an escape of the tubular content in the interstitial tissue and to the stimulation of an antigen-antibody reaction. These situations, however, are rare.

The study of the appearance of antispermatic antibodies in vasectomized men constitutes an argument in favour of this view.

The problem of the therapeutic approach is at present unsolved. It remains very difficult for the fact that the low rate of antibodies diminishes but very little the fertility of a couple. It is this the case in the above mentioned sub-

clinical situations which for many authors are even a negligible detail in the problem of the infertility.

Of all the approaches directed to correct a visible autoagglutination or an evident non-penetration of the spermatozoa into the glare in the course of invasion tests, none has the merit to lead to normalizations and with that to an elevated percentage of fecundations.

The vitamin C, small doses of corticoids or other hormonal treatments are useless.

In situations of heteroagglutination, of long interruptions of the sexual life or relations with condom for more than a year, are therapeutics whose efficacy remains to be proved.

Finally, a recent approach consisting in the administration of immuno-suppressors at high doses for a short time maybe promising, but it requests on one hand the greatest prudence in its applications and on the other evident positive results on large well studied series.

Autoimmunity and diabetes mellitus

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The time honoured separation of idiopathic diabetes mellitus into 2 types, indeed corresponds to 2 different pathological entities, which have in common glucose intolerance and the ensuing vascular complications. (Table 1).

In insulin dependent diabetes (IDD), usually but not always of youthful onset, extensive beta cell destructions explain the clinical and the therapeutic characteristics. IDD presents with a particular hereditary predisposition, associated with HLA markers. Epidemiological and experimental evidence favours the concept that a viral attack is responsible for the development of this form of diabetes in highly predisposed subjects (Creaghead and Nerup, 1977).

TABLE 1

The 2 main Diabetic Syndromes

<i>Characteristics</i>	<i>I.D.D. (yody; yoda)</i>	<i>N.I.D.D. (moda, mody)</i>
<i>Clinical</i>		
Onset	< 45 years, sudden	> 45 years, slow
Constitutional	Asthenic, lean	Hypersthenic, obese
<i>Pathological</i>		
β cells	destruction (insulitis)	(2/3 normal)
<i>Therapeutical</i>		
Effect of insulin	+	—
Effect of diet	—	+
Effect of tolbutamide	—	+
<i>Etiological</i>		
Virus	+ ?	—
I.C. Ab	+	—
<i>Hereditary</i>	+ (max 50%) HLA assoc.	++ no HLA assoc.

Common metabolic disturbances and ensuing complications

In the NIDD-syndrome, the onset of which usually takes place in adult life (MODA), but occasionally in youthful subjects (MODY), the beta cells remain granulated, no HLA genetic markers are observed although heredity plays a much more striking part than in the IDD.

The autoimmune processes are also quite different in these 2 syndromes. Whereas in NIDD, there is no evidence of a pancreatotropic autoimmune process, several findings suggest an autoimmune origin of ICC.

The first observation concerns lymphocytic insulitis with extensive and specific beta cells destruction, found in about 2/3 of insulin dependent diabetics, who died shortly after the onset of disease (Gepts, 1965-1976; Egeberg *et al.*, 1976).

The second finding is that of cellular autoimmune reactions against pancreatic extracts in subjects affected with IDD, mostly of recent onset (Nerup *et al.*, 1974).

Thirdly, four years ago, Botazzo *et al.*, 1974 using an immunofluorescent method, were able to show the presence of islet-cell antibodies (ICA_b) in the serum of IDD-patients.

Like the histological insulitis and the cellular immune reactions to islet tissue, the serological autoimmune reactions are present with a very high prevalence (up to 85%), only in the early stages of IDD. This prevalence drops to 50% in 4 weeks to 20% after 2 years (Lendrum *et al.*, 1976).

Although the pathology only shows B-cell destructions, the islet cell antibodies are initially directed against all types of islet-cells. By analogy with studies of other organ-specific autoimmune processes (Doniach, 1974; Pinchera, 1975), it is thought that the specific antigens to ICA_b probably consist of membrane lipoproteins present in the cytoplasmic vesicles, active in hormone synthesis and transport. As suggested by Del Prete *et al.*, 1977, it is possible that the ICA_b are primarily directed against the B cells but cross-react with the antigenic molecules of the other islet cells. These antibodies are supposed to be non-cyto-toxic. In non-diabetics, the presence of ICA_b does not seem to be associated with diabetic alteration of beta cell function (Tiengo *et al.*, 1977). However, it has recently been shown that the lymphocytes of newly diagnosed IDD-patients are able to induce diabetes when transplanted in athymic nude mice (Buschard *et al.*, 1978).

In IDD, besides the Langerhans islets reactions, other organ specific processes have been observed. The overlapping of several autoimmune reactions between idiopathic autoimmune diseases has been described by Feltkamp as early as 1966. The association of autoimmune thyroiditis, Addison's disease and pernicious anaemia has thus been considered as a marker of autoimmunity in IDD.

However, the incidence of autoimmune asymptomatic thyroiditis and gastritis (as detected by the presence of thyroglobulin and gastric antibodies in the serum) increases with the duration of the metabolic disorder (Whittingham *et al.*, 1971) and bears no relationship with the ICAb (Lendrum *et al.*, 1976). On the other hand, in contrast with current opinion, these organ-specific autoimmune reactions are also found with a high prevalence in elderly NIDD-patients (Bastenie *et al.*, 1972; Del Prete *et al.*, 1977).

Recent studies (Delespesse and Bastenie, 1978) have shown that amongst subjects aged 51 to 60 years, one or several circulating organ specific autoantibodies are encountered in 86% of those affected with IDD, 42% in those with NIDD and 11% of normal controls. These observations explain the well-known association of diabetes mellitus (IDD or NIDD) with idiopathic myxoedema (Andreani, 1974). Indeed, as we have shown, the latter condition is the end result of an autoimmune process of thyroid destruction, only detectable in its preclinical stages by thyroid antibodies increased basal TSH levels and reactions to TRH (Bastenie *et al.*, 1967, 1977).

To explain this association of diabetes with thyroid, gastric and adrenal cortex autoimmunity, we suggest that the diabetic metabolic process interferes with the normal immune mechanisms (Bastenie *et al.*, 1976).

The HLA lymphoblastic transformation test in female and male controls, shows a progressive reduction with age. In diabetic subjects, the DNA synthesis induced by PHA is markedly impaired, even in the youngest age groups and most so in poorly controlled IDD patients (Delespesse *et al.*, 1974; MacCuish *et al.*, 1974).

CONCLUSIONS

1. In IDD, HLA-associated autoimmune reactions (both humoral and cellular) are apparently important aetiological factors, responsible for the beta-cell destructions. It is probable that viral factors are at the origin of the transient immune reactions.

2. In NIDD, as well as in IDD, organ-specific autoimmunity often develops in relation with the severity and/or duration of the diabetes.

3. Diabetes, like old age is associated with impaired cell-mediated immunity, probably consisting in a functional defect of a subgroup of T cells (Duchateau *et al.*, 1976).

It is suggested that the diabetic disturbance like other debilitating diseases (Serafini, 1977) markedly favours the development of organ-specific autoimmune reactions.

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Autoantibodies to single endocrine cells in the human pituitary, pancreas and gut

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Development of a double fluorochrome, four-layer immunofluorescence technique in which the patient's serum is applied first and counterstained with FITC-goat-antihuman-Ig, while the third and fourth layers consist of appropriate rabbit hormone-antisera and rhodamine-labeled-goat-antirabbit-Ig, has made it possible to identify isolated cells showing cytoplasmic fluorescence attributable to specific antibodies directed to intracellular membrane antigens in anterior pituitary, pancreatic islets, gastric antrum and duodenum.

Pituitary

The two pituitary antibodies so far identified react with prolactin-cells and growth-hormone cells respectively. The first is found mainly in polyendocrine patients, especially those with idiopathic hypoparathyroidism, and in cases of partial pituitary deficiencies, while all cases of advanced panhypopituitarism have given negative results on human pituitary gland obtained at operations or fresh postmortems. Growth-hormone-cell antibodies have been found in a few young subjects with growth defects and isolated GH deficiency. It is suspected that further antibodies to other pituitary cells will be identified since a number of sera show IFL on cells which are neither lactotrophs nor somatotrophs.

Endocrine pancreas

1-4% of sera from patients with various autoimmune disorders contain antibodies that react specifically with either glucagon or somatostatin-cells in pancreatic islets. Half of these cross-react with the parallel endocrine cells in stomach mucosa or gut but others react only with the pancreas. These antibodies are not related to diabetes mellitus as are the islet-cell antibodies (ICA) having a common antigen in all the cells of the islets.

Gastric antrum

Chronic atrophic gastritis can affect mainly the body and fundal mucosa (Type A) when it is associated with gastric parietal-cell antibodies, especially

in patients with autoimmune endocrinopathies. This form of gastritis may progress to Addisonian pernicious anaemia. More recently a form of gastritis affecting only the antrum has been described and named Type B. Using human antrum and the double IFL technique we have been able to demonstrate antibodies reacting specifically with the gastrin-(G)-cells in 8/106 patients with biopsy proven antral gastritis. Gastrin secretion rates were diminished in these patients suggestive of selective loss of G-cells, possibly due to destruction by an autoimmune process.

Autoimmunity and chronic liver diseases

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For the past several years the immunological studies in acute and chronic inflammatory liver diseases were predominately concerned with:

1. The demonstration of hepatitis B virus antigens (and of immune reactions against these antigens) in sera and liver tissue;
2. The detection of autoimmune phenomena in sera and on isolated hepatocytes from patients with liver diseases;
3. The role of immune mechanisms in the pathogenesis of HBsAg negative and positive liver diseases;
 - 3.1. Immune response to liver membrane antigens;
 - 3.2. Immune response to hepatitis B virus antigens;
 - 3.3 Immune response to alcoholic hyalin and drugs;
 - 3.4. Immune complexes in liver diseases;
 - 3.5. Genetic predisposition for chronic active liver diseases;
4. The effect of immunosuppressive drugs in HBsAg negative and positive chronic active hepatitis.

The results of these studies give increasing evidence that the hepatic injury in acute and chronic active inflammatory liver diseases is mediated by immunological mechanisms.

SYMPOSIA
HYDRO-ELECTROLYTIC DISORDERS
IN INTERNAL MEDICINE

Disorders of body fluids and electrolytes in internal medicine

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During the past decade a multidisciplinary effort involving both basic and clinical scientists has produced a tremendous amount of data, with also conflicting findings with regard to the mechanisms underlying the Na and H₂O disorders in Internal Medicine.

The lenght of this symposium (one hour and 45 minutes) is clearly insufficient even to touch on all of the many aspects and problems involved.

It was very difficult for me to choose the points to emphasize in this 5 minutes introduction. In the end I chose the following two: *the first point* I would like to make is the relationship among the factors listed in the table in causing Na and H₂O retention, which is never produced by and excess of only one factor but by a combination of factors belonging to the different groups.

TABLE 1

<i>Humoral:</i>	Plasma concentrations of albumin, electrolytes (Na), glucose and urea
<i>Hormonal:</i>	Renin-angiotensin, aldosterone, vasopressin, natriuretic hormone and prolactin
<i>Hemodynamics:</i>	Central and hepatic venous pressure, renal blood flow
	Cardiac output, arterial pressure (blood volume)
	Effective (arterial) blood volume

Perhaps the most clear cut example illustrating the importance of this relationship is that of Aldosterone. An excess of Aldosterone or desoxycorticosterone alone produces, both in animals and in man, arterial hypertension with very mild fluid retention, but when this excess is combined with even a mild increase in caval venous pressure it produces Na and water retention with almost no change in blood pressure (Davis *et al.*, 1953). The explanation of this phenomenon has not yet been found, but it is certain that once found it may give a new insight into the understanding of the diseases we are going to discuss today.

The second point is the sequence of events occurring after the initial injury which have caused the alteration of Na and H₂O homeostasis.

A reduction of renal blood flow, for instance, is a common finding in many states of Na and water retention.

The sequence of events occurring during the days following the reduction in renal blood flow has been studied in details (Bianchi *et al.*, 1970), (Liard *et al.*, 1974), (Bianchi *et al.*, 1977).

These studies have also been carried out in conscious animals in order to avoid the interference of anaesthesia and surgery. The most striking finding was that, even though the degree of renal artery constriction remained constant, the type of modification of the indices of Na and H₂O metabolism changed from one day to another. In particular a sudden increase of plasma renin and blood pressure were observed with a mild decrease in CO indicating a rise on peripheral resistance (first phase), followed by an expansion of blood volume and an increase in CO while the renin tended to return toward the normal levels (second phase). Finally, all these factors tended to return to normal and the hypertension seemed to be accompanied only by an increase of peripheral resistance.

Does this sequence of events occur also in Man? Of course, in man is not possible to define so precisely the experimental protocol but we have found evidence for such a sequence (Bianchi *et al.*, 1970). In fact when renin, plasma and extracellular fluid volumes were measured in young patients with renovascular hypertension of probably recent onset, we were able to demonstrate that patients with plasma renin values within the normal range tend to have, on the average, significantly larger body fluid volumes than those with high renin. And, being this the most interesting point, successful surgical repair of the stenosis in patients with the expanded volumes normalizes both blood pressure and volumes. From these findings in animals and in man, the following two considerations seem to be pertinent for the topic of this symposium.

From the pathogenetic view point it is clear that we cannot use experiments carried out in acute animals, whether conscious or anaesthetized, to draw conclusions about the mechanisms regulating fluid homeostasis in the long run.

From the clinical view point: the same pathological situation may produce different body changes, depending on the phase in which we pick them up, and, of course, this may have also therapeutic implications.

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Water and salt metabolism in liver diseases

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Patients with acute or chronic hepatocellular disease retain salt and water as shown by weight gain, oedema and ascites. The aetiology of ascites complicating hepatic cirrhosis is complex. Reduction of serum colloid osmotic pressure, related to defective hepatic albumin synthesis, is combined with portal hypertension due to obstruction to portal flow to the liver and these are the prime movers. Hepatic lymph over-production follows the rise in intra sinusoidal pressure. The kidney attempts to compensate, the renin-angiotensin system is stimulated, so that aldosterone is produced in excess and sodium is conserved by the kidney. The defect in natriuretic hormone ("3rd factor") is operative in the proximal tubule.

An alternative "forward" "overflow" theory suggests that the renal sodium and water retention is the primary event. This is localised to the peritoneal compartment if the portal pressure and plasma osmotic pressure are altered to favour fluid retention in that area.

Ascites can be treated by correcting the prime movers. The portal hypertension can be relieved by a surgical portalsystemic shunt, but this carries an unacceptable operative mortality in such poor risk patients and the incidence of subsequent hepatic encephalopathy is high. The colloid osmotic pressure may be raised by infusion of salt-poor human albumin but this is very costly and the benefit is transient. Attention is therefore directed to the sodium and water retention.

If ascites is gross, the patient is put to bed rest in hospital. He is weighed daily at the same time and a chart kept. Serum electrolyte and urea levels are estimated. 24-hour urine output is measured and, if possible, the sodium and potassium concentrations in it. Fluid intake is restricted to one litre. In hepatic ascites, renal sodium retention is very avid, the 24-hour urinary level usually being less than 10 mEq daily. In contrast to milder forms of fluid retention, diuretic therapy is not enough and dietary sodium must be restricted to less than 22 mEq daily. This is achieved by cooking without salt, by avoiding foods containing excess sodium and concentrating on a vegetarian diet with salt-free bread, butter and rice. Flavouring is achieved with garlic, mustard and sodium-free salt substitutes.

Four days are allowed for equilibration. During this period potassium sup-

plements (80 mEq daily) are given as the chloride. If a diuresis has not ensued a start is made with potassium-sparing agents such as spironolactone (100 mg) or amiloride (10 mg). After 24 hours, the doses of these drugs may be increased or a drug such as frusemide (30 mg) or bumetamide (3 mg) is added. The doses of these various diuretics are titrated according to the patient's response as judged by daily weighing and urinary output. A daily fall in weight of 0.5 kg or an increased diuresis of about 500 ml is regarded as ideal. Meticulous attention must be paid to the dangers of over-diuresis, to potassium depletion and azotaemia (twice weekly serum estimations) and to the development of encephalopathy (check for flapping tremor daily).

Formal paracentesis is performed only if the patient is suffering from pain and dyspnoea due to tense ascites. A small sample (about 10-20 ml) of ascitic fluid is, however, always tested. The protein content is analysed. In most forms of cirrhosis, the value is 1-2 g %. A high value suggests intrinsic disease of the peritoneum (infection or tumour) or the Budd-Chiari syndrome. High values (greater than 2.5 g/100 ml) are also found sometimes in uncomplicated cirrhosis (Sampliner and Iber, 1974). Pancreatic ascites, found predominantly in alcoholics with pancreatic pseudocysts or ruptured pancreatic duct, is also associated with a high ascitic protein content (Donowitz *et al.*, 1974). Spontaneous bacterial peritonitis is a frequent complication of hepatic ascites, especially if the liver disease is decompensated (Conn, 1976). It should be suspected if the WBC count in the fluid is 300-500/mm³, with polymorphs 75-100/mm³. The protein content may not necessarily be increased. Direct, gram-stained smears of the centrifuge deposit may be useful. In two-thirds, the infection is gram negative. A search should also be made by direct smear and culture for acid fast, tuberculous, infection.

In the majority of patients, at least 90%, the ascites will respond to the dietary and dietetic regime outlined above. In some patients, however, ascites is gross and diuretics have to be pushed to extreme doses in attempts to produce an increase in urine output. In some of these refractory patients, there is particular danger, when the intravascular department is further depleted by diuretics that functional renal failure ("hepato-renal syndrome") develops (Kew, 1972). In these resistant cases, other lines of treatment have to be considered. Further diuretics will increase the likelihood of the development of the hepatorenal syndrome. Large paracenteses are contraindicated, except for urgent relief of tense ascites causing pain and dyspnoea. Serum sodium levels fall and blood urea values rise. Infusion of concentrated saline will only lead to pulmonary oedema. Restriction of fluid intake is indicated, but will not be tolerated by the patient who will go to any extreme to slake his thirst. He will drink from the toilet basin, from the water tap or even from the hot water bottle.

The infusion of dextran or salt-poor albumin expands the plasma volume and may initiate a diuresis. The unaltered ascitic fluid has been reinfused and this also can be effective. An improvement on this technique is to pass the ascitic fluid via a peritoneal dialysis catheter over an ultra-filter fitted with a special membrane allowing the passage of fluid and crystalloids with a molecular weight of less than 50,000 but retaining the protein concentrate. This is returned to the patient intravenously. The plasma volume expands and renal blood flow improves. A suitable apparatus with pump and ultra-filtre has been developed in France and has been used extensively (Levy *et al.*, 1971). Up to 13 litres of ascites can be removed in a 21-hour ultrafiltration (Parbhoo *et al.*, 1974). The weight loss is even greater than would be accounted for by the volume of fluid ultra-filtered, because urine flow increases during and after the procedure. The patient is more responsive to diuretics. If the ascitic fluid protein is high, the membrane will clog. The technique is unsuitable for malignant ascites. Infection of the ascitic fluid is also a contra-indication. The procedure is expensive, but this must be weighed against the cost of a longer stay in hospital. Transient pyrexia, pulmonary oedema and intraperitoneal haemorrhage have been reported as complications (Moult *et al.*, 1975). The reinfusion rate must be monitored, central venous pressure rises in almost all instances (Villeneuve *et al.*, 1977). Fluid overload may lead to heart failure and may precipitate variceal haemorrhage. A mild consumptive coagulopathy can develop (Villeneuve *et al.*, 1977). Moreover, the method may be only palliative as ascites can reaccumulate rapidly.

A successful use of the reinfusion technique is illustrated (Fig. 1). This 48 year-old man with hepatitis B antigen positive cirrhosis had not had a diuresis after 200 mg spironolactone and 120 mg frusemide therapy daily for two weeks. His blood urea level was rising. The reinfusion pump was used over 48 hours. Approximately 20 litres of ascitic fluid were filtered. The body weight fell 34 kg. There was a profound diuresis and urinary sodium excretion increased.

LeVeen and colleagues from Brooklyn New York, have devised a peritoneo-venous shunt which removes peritoneal fluid more continuously over many months (LeVeen *et al.*, 1974, 1976). The peritoneal cavity is drained by a long perforated plastic tube that reaches into the pelvis. This connects with a special pressure-sensitive valve lying extraperitoneally and deep through the abdominal muscles. This again connects with a silicone rubber tube which passes subcutaneously from the abdominal wound, towards the neck and so into the internal jugular vein. The end of the tube is left in position in the superior vena cava. The operation is possible under local anaesthesia, but the insertion of the subcutaneous tube can be painful and a light general anaesthetic is often necessary. Antibiotic cover is provided. A loss of ascitic fluid is minimised during surgery. Meticulous closure of the abdominal wound will reduce the risk of leakage which

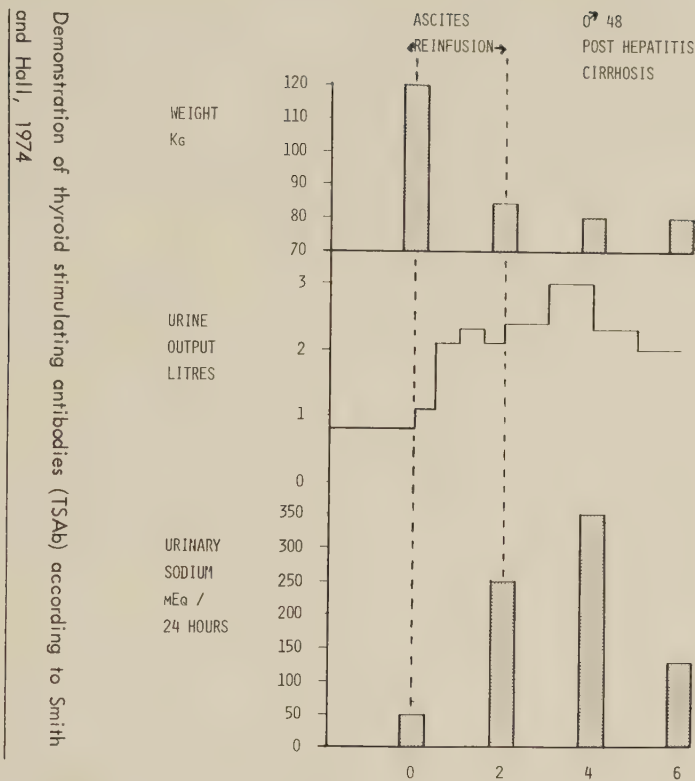


FIG. 1. Successful use of the ascites-concentration-reinfusion method (Rhodiascit) for the treatment of ascites in a 48 year old man with post hepatitis cirrhosis.

can be responsible for infection. As the diaphragm descends during inspiration, the intraperitoneal fluid pressure rises, whereas that in the intrathoracic superior vena cava falls. This results in a pressure differential of about 5 cm H₂O. Respiration provides the force which opens the valve and propels the fluid into the superior vena cava. The venous tube remain patent only when its interior contains ascitic fluid. The specially designed pressure-sensitive valve totally prevents entry of blood into the venous tubing. Between 1973 and 1976, LeVeen and his group (1976) have treated 62 patients with ascites of various aetiologies and there are 210 other instances reported in the literature. Ten of the 22 patients treated after 1973 survived at least 12 months (Wapnick *et al.*, 1977). The technique may be particularly useful in the Budd-Chiari syndrome. The exact mortality and morbidity is difficult to determine from the literature. There is however no doubt that some patients do exceedingly well with improved nutrition, rise in serum albumin levels, increased urinary flow and control of ascites.

Successful use of the LeVeen shunt is illustrated (Fig. 2). This 56-year old patient with alcoholic cirrhosis had had multiple hospital admissions in the past year for treatment of ascites uncontrolled by diet and diuretics. After insertion of the peritoneo-jugular shunt there was an immediate negative fluid-balance, urinary sodium increased and weight dropped 15 kg in 6 days. The weight stabilised but after 12 days the patient gained weight, representing im-

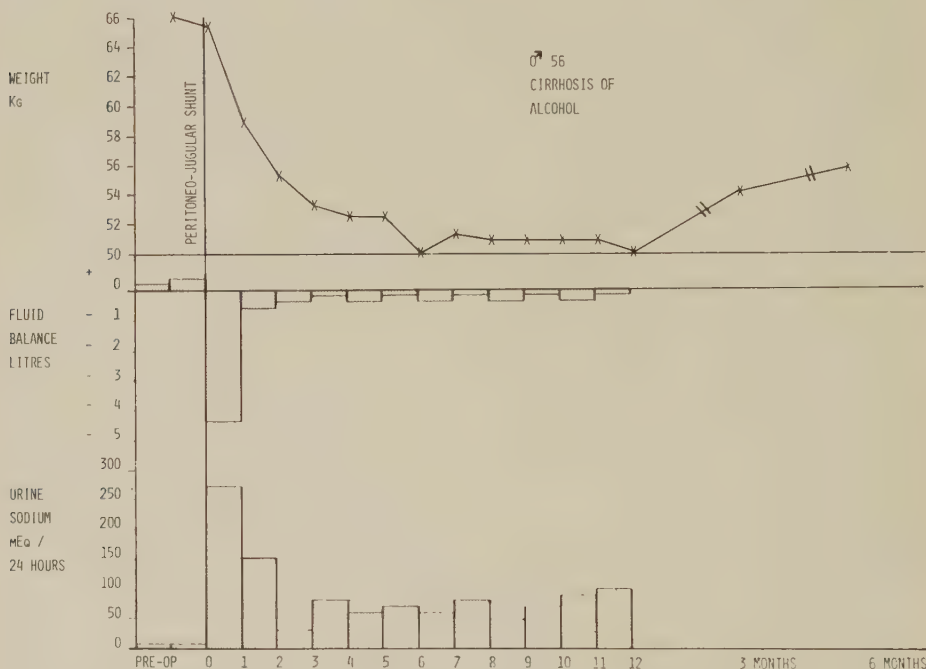


FIG. 2. Successful use of the peritoneo-jugular shunt (LeVeen) in the treatment of ascites in a 56 year-old man with cirrhosis of the alcoholic.

proved nutrition. At three months he had gained 4 kg and at six months 5.6 kg. He needed only modest amounts of diuretics. He had not required a hospital admission.

The LeVeen shunt may have a beneficial effect on renal function. Serum renin, angiotensin and aldosterone levels decrease (Wapnick *et al.*, 1977; Greig *et al.*, 1977). It has even been used to manage the hepato-renal syndrome (Fullen, 1977). Thirty cirrhotic patients with refractory ascites and functional renal failure have been relieved (Wapnick *et al.*, 1977). Complications include fever, leakage of peritoneal fluid, blocked shunt, infections, both subcutaneous and

systemic, and bacterial endocarditis. In every instance, a mild disseminated intravascular coagulation can be detected and occasionally this may be severe and fatal. There is a risk of embolus if an abdominal organ perforates with the shunt in position.

If the LeVeen shunt is used in a patient with poor hepato-cellular function, results can be disastrous. Such a patient is illustrated in Figure 3. This 23 year-

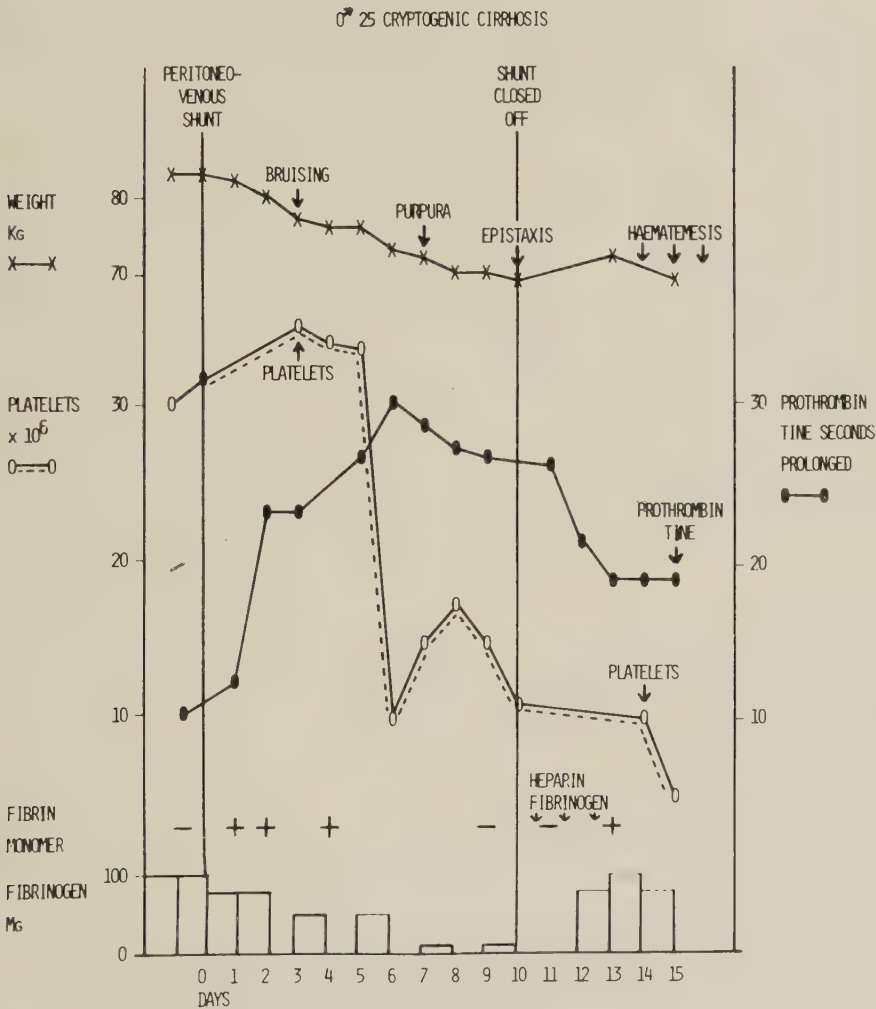


FIG. 3. The use of the peritoneo-jugular shunt in a 25 year-old man with cryptogenic cirrhosis and poor hepato-cellular function was followed by a disseminated intravascular coagulopathy. Platelets, prothrombin and fibrinogen fell and fibrin monomers appeared in the blood. These persisted following closure of the shunt.

old man with cryptogenic cirrhosis had gross refractory ascites. His serum bilirubin level was 10 mg, serum albumin 2.5 g, platelet count 30,000 and the prothrombin time prolonged 10 seconds over the control. Insertion of the LeVein shunt was followed by bruising, purpura and epistaxis. Circulating platelets fell precipitously despite platelet infusions. Circulating fibrinogen levels fell and fibrin monomers could be detected. On the 10th day, the shunt was closed off, but this did not halt the consumptive coagulopathy. Heparin, fibrinogen and fresh frozen plasma proved ineffective therapies and he died on the 15th day after the shunt had been inserted. The disseminated intravascular coagulopathy is thought to be due to thrombin generated by the rapid infusion of one or more thromboplastin-like materials present, but not yet identified, in the ascitic fluid.

With modern diuretic and dietary regimes, such an operation as the LeVein shunt should rarely be necessary. It may, however, be useful in those who cannot co-operate with diet and diuretics, the alcoholic who insists on continuing alcohol and those coming from countries where medical services are insufficient to handle the management of fluid retention and salt-free diets. It needs further evaluation in the early treatment of the hepato-renal syndrome.

The treatment of ascites complicating hepatic cirrhosis gives most gratifying results in almost all instances. The patient feels that at least some action is taking place in treating what is usually end stage cirrhosis. The patient is relieved of his discomfort. Whether life is prolonged by these various measures however, has never been established.

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Water and salt metabolism in heart diseases

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The pathogenesis of water and salt metabolism in congestive heart failure (CHF) certainly involves hemodynamic and hormonal factors; several aspects of this syndrome still need to be clarified. Among these, the following topics are noteworthy: a) how myocardial failure triggers the sequence of hemodynamic, renal, biochemical and hormonal events that, eventually, lead to water and salt retention; b) which is the nature of the hypovolemic stimulus that induces the kidney to behave paradoxically, very much like the kidney in the salt and water depletion; c) what is the role played by different hormones (i.e. aldosterone, ADH, natriuretic hormone) in the genesis of water and salt retention. It has to be underlined that the hypovolemic signal that represents the first step leading to edema, i.e. the effective arterial plasma volume (EAPV), is a hemodynamic factor which to this day has remained an unmeasurable quantity. Therefore, its absolute changes and the variation in respect to those of fluids in the different districts of the body, remain unknown.

Hyperaldosteronism: several informations seem to demonstrate that it represents a secondary event, both for time of appearance and for pathogenetic importance, in water and salt retention associated with CHF. In particular, highly suggestive of its secondary role are the following data: 1) an elevated aldosterone concentration is not observed in the initial period of CHF (1); 2) water and salt retention and edema formation have been observed in adrenalectomized patients with CHF; 3) edema is usually absent in patients with primary hyperaldosteronism; 4) the administration in normal subjects of high doses of aldosterone fails to produce edema, as a consequence of the so-called "escape" phenomenon, i.e. the increase of sodium excretion within a few days from the beginning of aldosterone administration (2, 3, 5) aldosterone antagonists, such as spironolactone, are unable, when administered alone, to prevent sodium and water retention and edema in patients with CHF.

Antidiuretic hormone (ADH): it is still unclear whether an increased concentration of this hormone is constant and present from the initial phase of

cardiac failure, and, also, what is its real role in the genesis of water and salt retention. Actually, although ADH concentration is found always elevated in CHF, edema can develop also when cardiac failure is present in patients with diabetes insipidus.

Kidney hemodynamics: changes in kidney function induced by altered renal hemodynamics, may play a role in the development of water and salt retention which is one of the features of cardiac failure, that usually appears before changes in glomerular filtration rate and in venous pressure are observed (5). It is conceivable, however, that, while during the initial phase of heart failure, kidney function is still preserved under basal condition, it is unable to meet the increased requirements produced by oral water and salt loading. In particular, this could interest some of those parameters of kidney function that are usually measured by methods biased by relatively large errors. As an example, methods currently used to measure glomerular filtration rate (GFR) are connected with an error ranging from 5 to 10%, while changes of only 1% in tubular reabsorption may affect significantly kidney function. As a consequence, methods currently available to evaluate tubular reabsorption, i.e. renal clearances, are not very sensitive and, thereby, severely limited. Therefore, many of the doubts still existing regarding the intricate pathogenetic mechanisms leading to water and salt retention in CHF, have to be referred at least in part to inappropriate study methods. In particular, in the initial phase of CHF most of hemodynamic, hormonal and renal determinants can be normal under basal condition, while the same determinants are affected deeply under dynamic conditions; this is what probably takes place following water and salt absorption, when changes in water and salt exchange induce the intervention of the various compensatory mechanisms that in normal subjects rapidly restore normal intra and extracellular body fluid volume and osmolarity, oncotic pressure and electrolyte balance. Therefore, it seems reasonable that the intimate pathogenesis of water and salt retention in CHF could be ascribed to changes of the delicate mechanisms that physiologically regulate water and salt balance and osmotic homeostasis of body fluid, following water and salt assumption. Unless anatomic and functional changes of kidney tissue are present, due to chronic congestion or to inappropriate use of drugs (diuretics, in the first place), the kidney is able to adapt its function to the different hemodynamic and humoral conditions that follow the assumption of water and sodium chloride (this latter being the salt most widely represented in the food and the one mostly responsible for water and salt retention) in hypotonic, isotonic or hypertonic concentration, as compared to body fluids.

When comparing in normal subjects and in patients with CHF changes in ventricular function curves (Frank-Maestrini-Starling curves), in kidney function

and ADH and aldosterone blood concentration, induced by oral loading of water and of NaCl isotonic solution, data obtained are deeply different in the two groups. In normal subjects, following the oral loading of 1000 ml of water, changes in left ventricular function curves remain within normal limits, since left ventricular end diastolic pressure remains almost unchanged, while stroke volume rises significantly (Fig. 1). Meanwhile, there is an increase in renal plasma

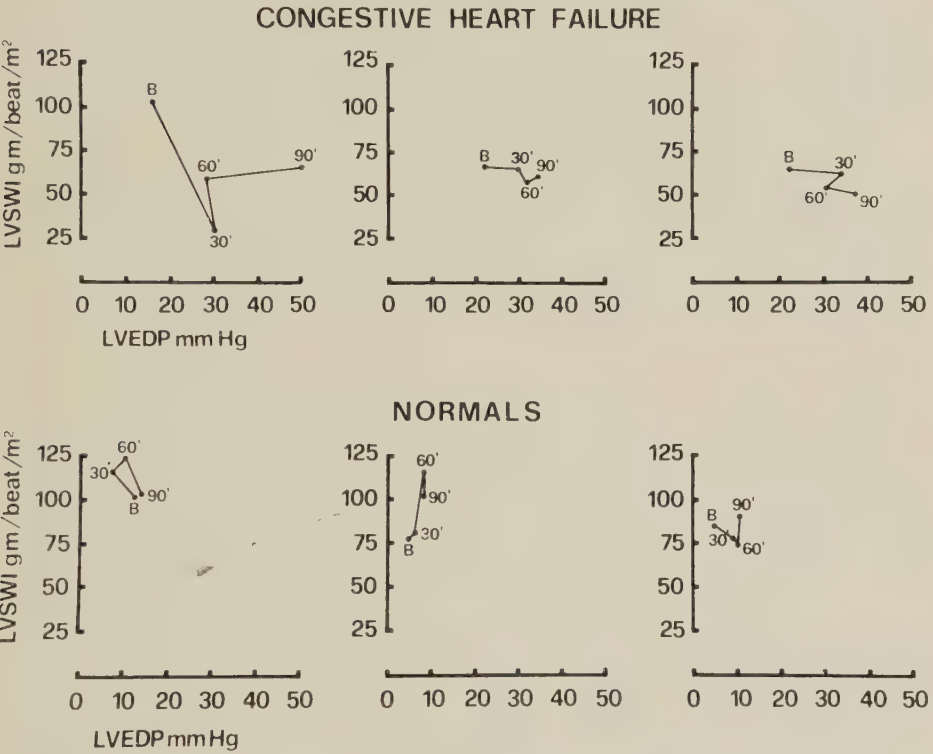


FIG. 1. Left ventricular function in three patients with congestive heart failure (top) and three normal subjects (bottom). In normal subjects following 1 liter water loading left ventricular end diastolic pressure (LVEDP) changes slightly while left ventricular systolic work index (LVSWI) increases. In contrast in patients with CHF water loading is followed by an increase in LVEDP while LVSWI either decreases or remains unchanged.

flow (RPF) and in GFR, without appreciable changes in glomerular filtration fraction (FF) (Fig. 2), while plasma osmotic pressure, plasma protein, sodium, ADH and aldosterone concentration and PRA are reduced. These events are responsible for the fast elimination of the assumed water, which starts within 15 to 30 min from the loading and is completed within about 90 min, with the

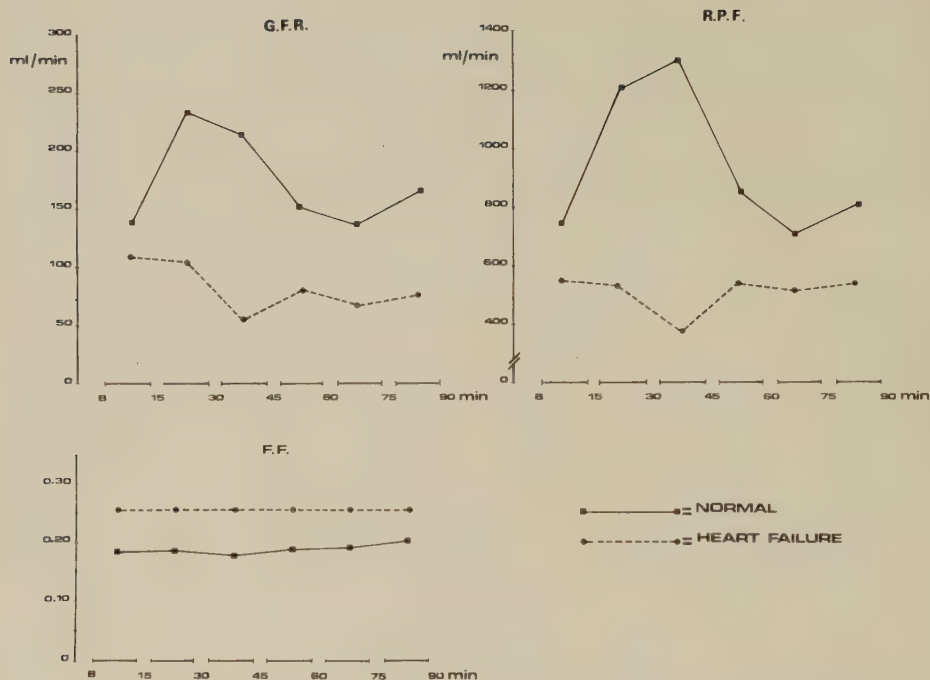


FIG. 2. Renal plasma flow (RPF) and glomerular filtration rate (GFR) changes in normal subjects (■—■) and in patients with CHF (●---●). In normal subjects GFR and RPF are both increased by water loading, and, thus, filtration fraction remains unchanged. On the contrary, in patients with CHF water loading decreases both GFR and RPF and FF remains constant, although at levels higher than in normal subjects.

production of hypotonic urine (Fig. 3). The sequence of events which eventually leads to the complete elimination of the ingested water is probably as follows (Fig. 4): the water loading induces an increase in total blood volume and in venous return; since heart performance is normal, this results in an increase in cardiac output and RPF. This latter factor is mostly responsible for the increased urinary output, for it affects GFR and reabsorption in the proximal and partially also in the distal tubular tract. In the glomerules, an increase in GFR is noted, due to the reduction in oncotic pressure gradient secondary to the fall in oncotic pressure in the glomerular capillaries. This reduced oncotic pressure, in turn, is due to hemodilution, and also to the increase in RPF (6). It is still unclear whether the increase in GFR play an important role in the augmented urinary output secondary to water loading. This could be the case if the actual filtration rate exceeds a maximal critical volume and the so-called glomerular-tubular balance can not work fully (by this mechanism, i.e. the glomerular tubular balance,

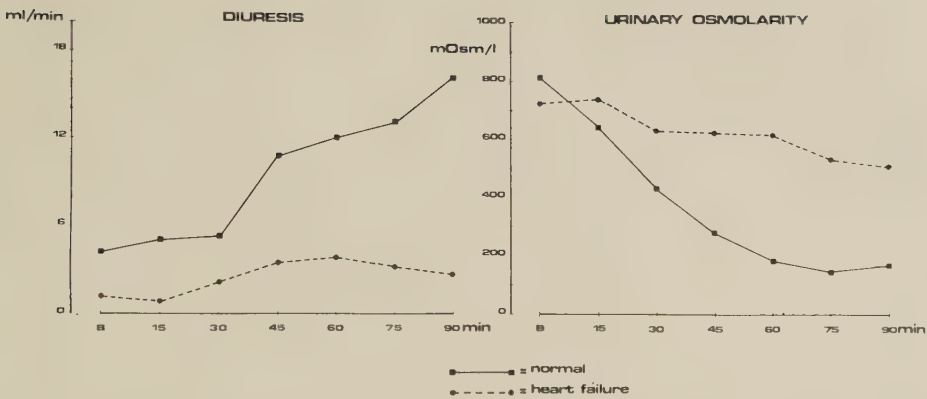


FIG. 3. Diuresis (ml/min) and urinary osmolarity (mOsm/l) after water loading in normal subjects (■—■) and in patients with CHF (●---●). In normals following water loading increases and urinary osmolarity decreases; in contrast, in patients with CHF, urinary output diuresis is only slightly elevated and urinary osmolarity does not change.

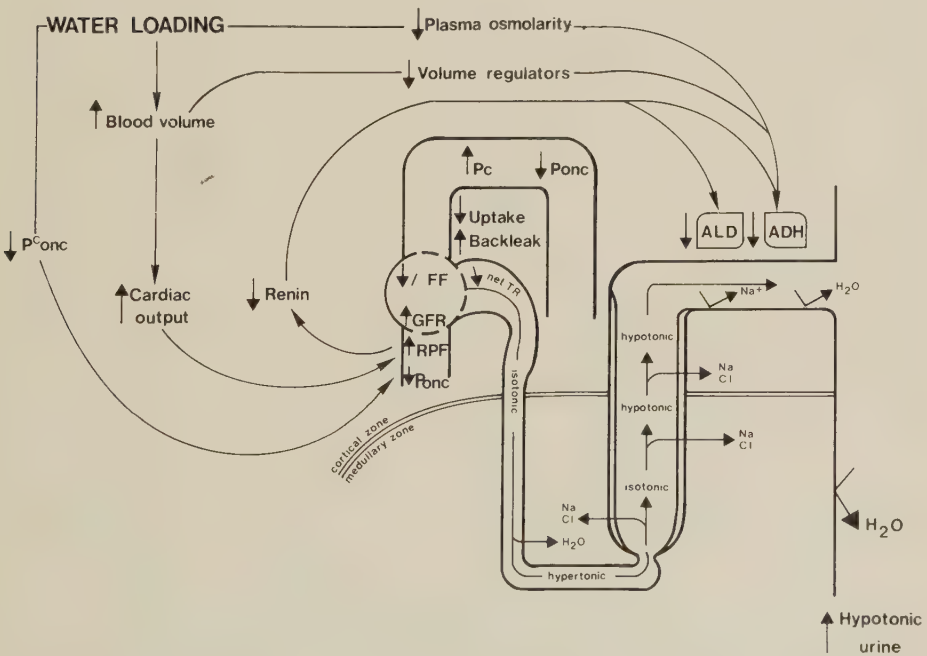


FIG. 4. Schematic representation of mechanisms that in normal subjects regulate the fast excretion of the water ingested. (For explanation see text).

within certain limits, for any given change in GFR, a similar change in tubular reabsorption takes place). In any instance, however, it is well established that the increase in RPF and the reduction in plasma oncotic pressure, both reduce the net reabsorption by proximal tubules. This is due to the increased blackleak of the renal interstitial fluid in the proximal tubule that follows the reduction in fluid uptake by peritubular capillaries. This event is the consequence of the increased hydrostatic pressure, secondary to the rise in RPF and also to the reduction in plasma oncotic pressure caused by hemodilution and by the increased RPF. Thereby, a reduction of the net reabsorption takes place at the level of the proximal tubule, which, in turn, implies an increased pre-urine delivery to the distal segment of the nephron; the volume of this delivery is the main factor determining urine volume concentration, provided that the effects of ADH and aldosterone on the distal segment of nephron remain constant (5-8). The pre-urine from the proximal tubule arrives to the descending part of the Henle's loop, which is located in the renal medulla, where the highest osmotic pressure is found. Here, pre-urine becomes hypertonic for the diffusion of water from the nephron to the medulla tissue, due to the osmotic gradient. The ascending part of the Henle's loop is water-proof, independently from the presence of ADH; therefore, in this segment the pre-urine becomes hypotonic, since an active reabsorption of chloride takes place, which implies a passive sodium reabsorption (9). It is in this part of nephron, the so-called dilution segment, that, under physiologic condition, the reabsorption of 15 to 20% of the filtrated sodium chloride takes place, and this value may increase by two or three times. Aldosterone does not influence sodium re-uptake at this level. By the mechanism described, in the ascending segment of Henle's loop, the free water is found, i.e; the preurine free of electrolytes. It remains in the tubular lumen, while sodium chloride in the medulla, induces hyperosmolarity in this area and, therefore, the water extraction from the ascending segment of the Henle's loop and, when ADH is present, also from the collecting ductus. In the distal tubule, in the cortical zone, from 5 to 10% of the filtered sodium is reabsorbed; it is an active process, influenced by aldosterone, obtained by exchanging this ion with potassium and hydrogen. Therefore, aldosterone does not influence directly potassium and hydrogen excretion, however, the amount of these ions that are excreted depends solely from the amount of sodium reabsorbed under the influence of aldosterone. As a consequence, should the amount of sodium arriving to distal part of the nephron be small, the excretion of potassium and hydrogen will remain unchanged, even in presence of very high aldosterone concentration (10-14).

Finally, in the terminal part of the nephron (collecting tubule and duct) located in the medulla, under the influence of aldosterone and probably also of other substances (natriuretic hormone, prostaglandins, renin-kallikrein system)

another 5% of the filtered sodium is absorbed (15, 16). ADH exerts its action only at the level of the collecting duct, where the permeability to water increases under the action of the hormone, while the water absorption itself is determined by the hyperosmolarity in the medulla. In normal subjects, as a consequence of the water loading, there is a reduction in water reabsorption in the distal segment of the nephron, due to the fall in plasma ADH concentration (Fig. 5).

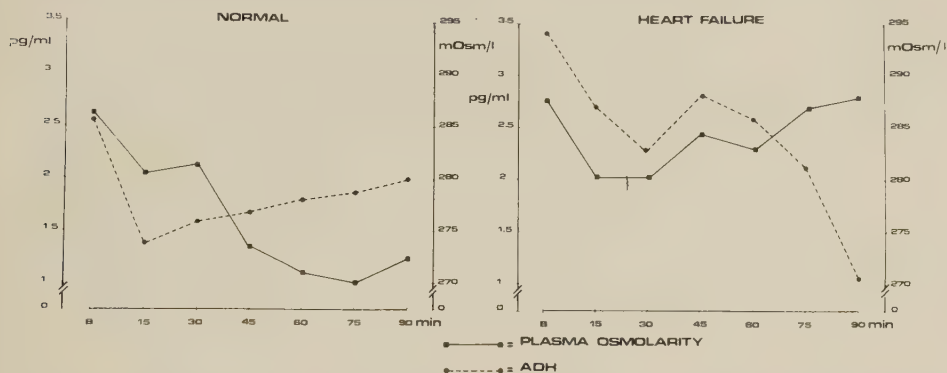


FIG. 5. Effect of water loading on plasma osmolarity and ADH concentration in normal subjects and in patients with CHF. Note that normals at any time have lower plasma ADH concentration and osmolarity as compared to patients with CHF.

This reduction in ADH concentration, confirmed by the increase in free water clearance, (Fig. 6) can be determined by several causes, among which the most noteworthy are: 1) the reduction in plasma osmotic pressure and the consequent inhibition of the Verney's hypothalamic receptors, controlling ADH secretion (Henry-Gauer reflex) (17-20); 2) the reduction in PRA, due to the increase in RPF, which has been shown in our laboratory to play an important role in the regulation of ADH secretion (Fig. 7) (21-23). Aldosterone concentration, after a short increase, is reduced, probably for the fall in PRA (Fig. 8); therefore, it seems that, following water loading, aldosterone secretion is influenced by opposite stimuli, with the inhibiting stimuli prevailing.

In conclusion, in normal subjects water loading does not modify the hemodynamic factors and the Starling's forces that regulate the reabsorption in the proximal tubule, the amount of pre-urine arriving to the ascending part of the Henle's loop is increased and, therefore, a larger amount of hypotonic pre-urine is produced; meanwhile, for the reduced ADH plasma concentration, the water reabsorption in the distal segment of the nephron is reduced, with the eventual result of the excretion of a large volume of hypotonic urine (Fig. 3). In patients with cardiac failure, diuresis after a water loading depends on the severity of

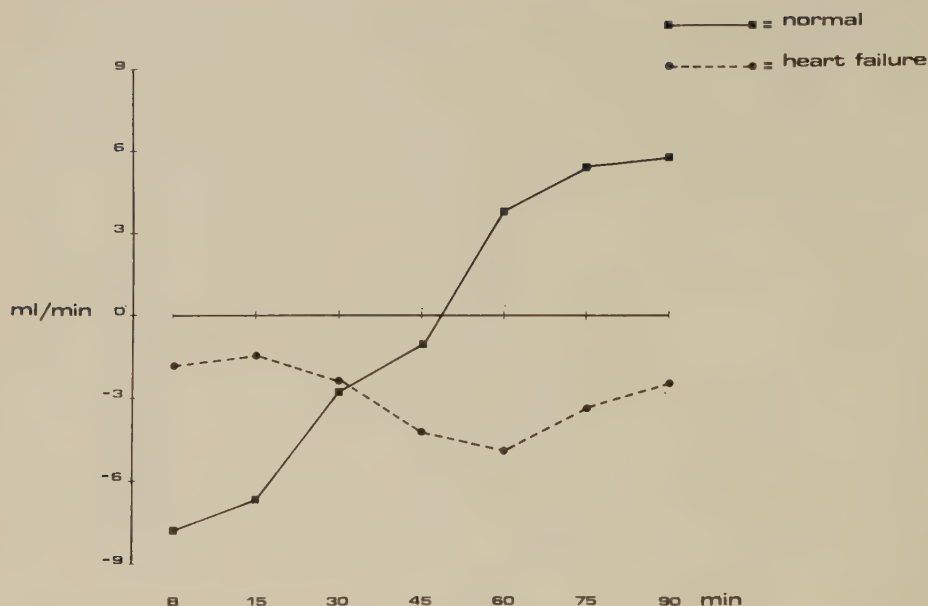


FIG. 6. Free water clearance in normal subjects and in patients with congestive heart failure after water loading. In normal subjects a higher free water clearance is observed as compared to patients with CHF.

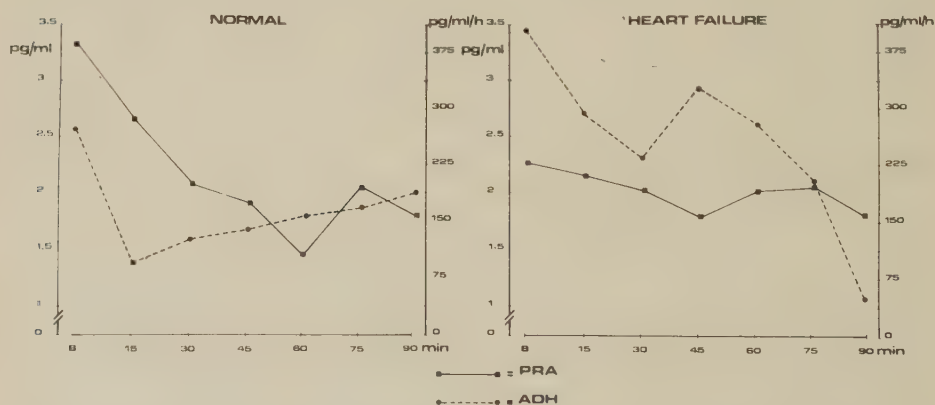


FIG. 7. Effects of water loading on plasma renin activity (PRA) and ADH plasma concentration in normal subjects and in patients with CHF. Note that in normals PRA falls sharply, as a result of the increased RPF. In patients with CHF, where RPF does not increase, also PRA remains unchanged.

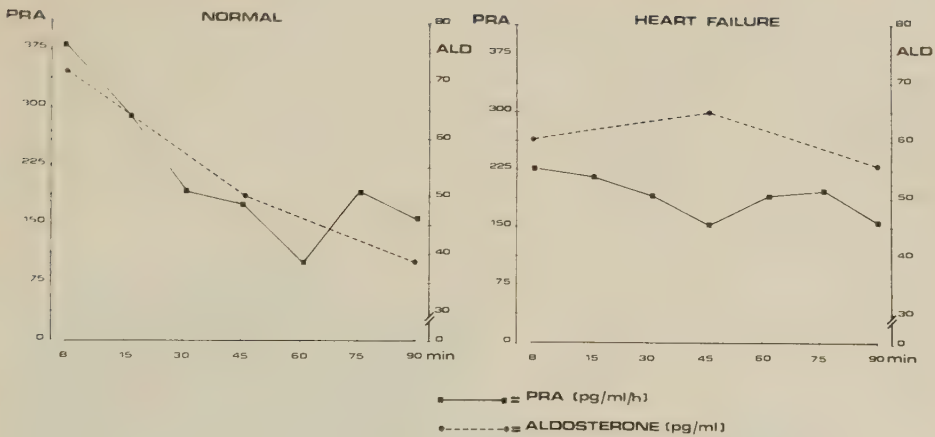


FIG. 8. Effects of water loading on aldosterone blood levels and plasma renin activity (PRA) in normal subjects and in patients with CHF. Note that in patients with CHF water loading affects only slightly these parameters.

failure. However, it has been generally recognized that a water loading induces in these patients a decline in cardiac output (Fig. 1) which leads to reductions in both RPF and GFR. In contrast, FF, which under basal conditions is higher than in normal subjects, remains unchanged after a water loading (Fig. 2). As a consequence of these hemodynamic events, the net reabsorption at the level of proximal tubule increases or at least does not change. At the level of glomerular capillaries, the decline in RPF leads to a fall in hydrostatic pressure, which counteracts the decrease of oncotic plasma pressure induced by hemodilution. Therefore, we may assume that the reduction of RPF in peritubular capillaries induces a decrease in hydrostatic pressure higher than the reduction in oncotic pressure. As a consequence, Starling's net forces of peritubular capillaries may remain unchanged or even decrease; consequently, the interstitial fluid uptake by peritubular capillaries may result unmodified or reduced, respectively; net tubular reabsorption may not change or diminish, and finally, delivery to the distal segments of the nephron may remain unchanged or diminish (24-26).

Both these latter events do not consent the increase in urinary volume, even if plasma ADH concentration falls. It has been reported that the reduction of GFR below critical level may induce contraction of urinary output also in patients affected by diabetes insipidus, i.e. with deficiency of ADH (Fig. 9) (4, 27). Since under this condition the collecting duct is not completely water proof, the remarkable pressure gradient between the intraluminal fluid, which is hypotonic, and the medullar interstitial fluid, which is hypertonic, induces water backleak

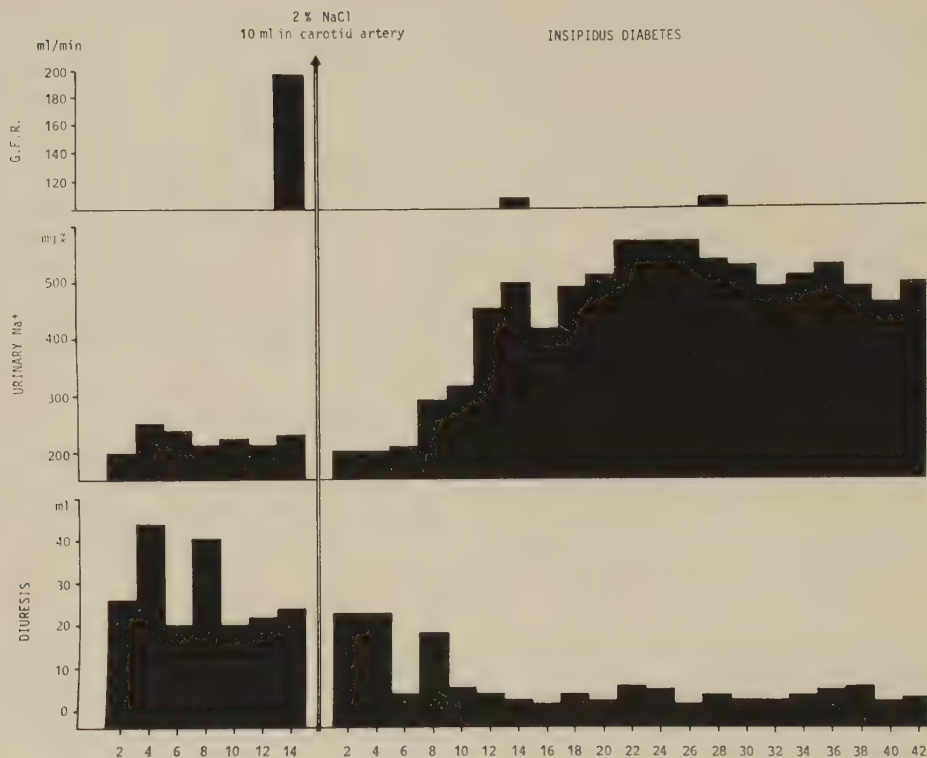


FIG. 9. Effects of hypertonic solution oral loading on GFR, sodium excretion and on urine output in patients with diabetes insipidus. Hypertonic solution administration decreases GFR and diuresis and increases sodium excretion. This event is probably due to the incomplete water impermeability of the collecting duct even in the absence of ADH; therefore the gradient between hypotonic endoluminal fluid and hypertonic medulla allows the water backleak.

from the duct lumen to the medulla. The amount of water which backleaks to the medulla is related to the volume delivered to the collecting duct.

If this volume is sufficiently reduced, the production of hypertonic urine is possible also in absence of ADH. The response of ADH and aldosterone to water involves remarkable differences between normal individuals and patients in heart failure. In the latter, ADH values under basal condition are significantly higher than in normal subjects, probably because of higher plasma osmotic pressure and/or reduced renal blood flow. The water loading reduces ADH in normal subjects and in patients with heart failure, however, in the latter, plasma levels of this hormone remain always higher than in normal individuals (Fig. 5). This is probably related to the fact that in patients with

heart failure the decrease in PRA following water loading is lower than in normals. This event depends on RPF, which does not increase as in normal subjects (Fig. 2).

However the behaviour of plasma osmotic pressure, which also influences ADH plasma levels, is different in cardiac patients as compared to that of normal subjects (Fig. 5) (29, 30). This is likely due to the venous congestion which occurs in the liver district in cardiac patients. As a consequence, the decrease in plasma osmotic pressure is mitigated and, thus, the entity of the stimulus which regulates the ADH excretion through the osmotic receptors, is reduced. This hypothesis seems to be confirmed by our studies both in animals and in humans. In animals, we have observed an increased osmolarity in blood samples collected by vena porta and hepatic veins, as a consequence of the venous congestion induced by a balloon inflated into the inferior vena cava (Fig. 10). Similarly, following water loading, the increase in liver blood flow and the reduction in

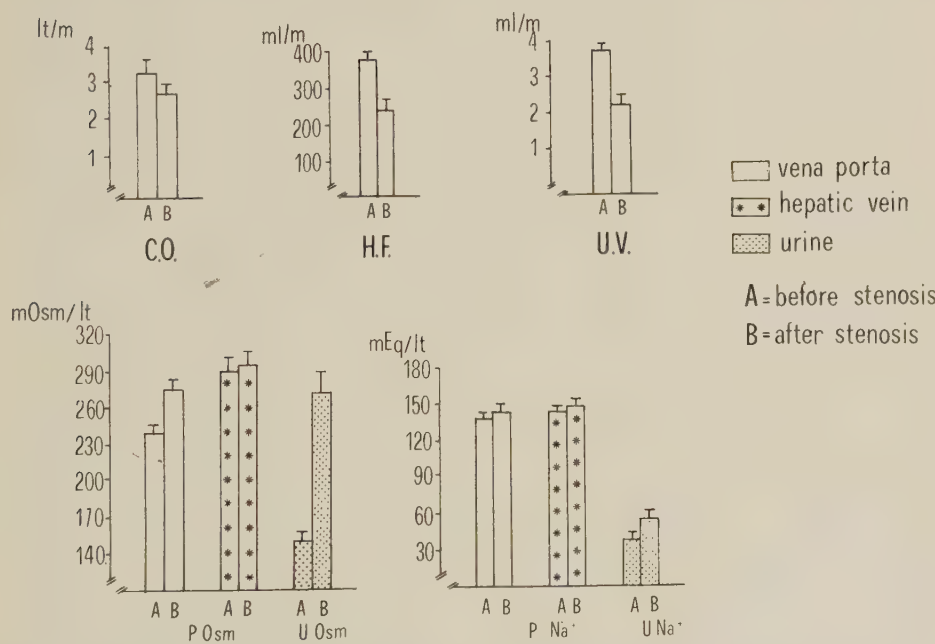


FIG. 10. Effects of inferior vena cava stenosis, obtained by inflating a balloon during oral water loading in dogs, on cardiac output, hepatic flow and urinary volume (upper panel) and on (lower panel) osmolarity and sodium concentration in the blood drained from the vena porta (solid columns), hepatic vein (columns with asterisks) and in urine (dotted columns). CO: cardiac output; HF: hepatic flow; UV: urinary volume; POsm: plasma osmolarity; UOsm: urine osmolarity; PNa⁺: plasma sodium concentration; UNa⁺: urinary sodium concentration; A: before inferior vena cava stenosis; B: after inferior vena cava stenosis.

the osmotic pressure of blood collected from the hepatic veins, both are less evident in patients with heart failure, as compared to normal subjects (Fig. 11) (23, 31, 32). In patients with CHF, about 90 min following water loading, a reduction in ADH plasma concentration takes place, in contrast to what observed in normal subjects, in whom the basal concentration is restored; this event is probably due to the intense osmotic receptors stimulation which follows the water entrance in the cells. In spite of this fall in ADH plasma concentration, to levels lower than those observed in normal subjects under basal condition, urine output and free water clearance remain substantially unchanged, since, for the altered hemodynamic, the amount of pre-urine reaching the ascending part of the Henle's loop, is very little (Fig. 3-6). As a consequence, at the level

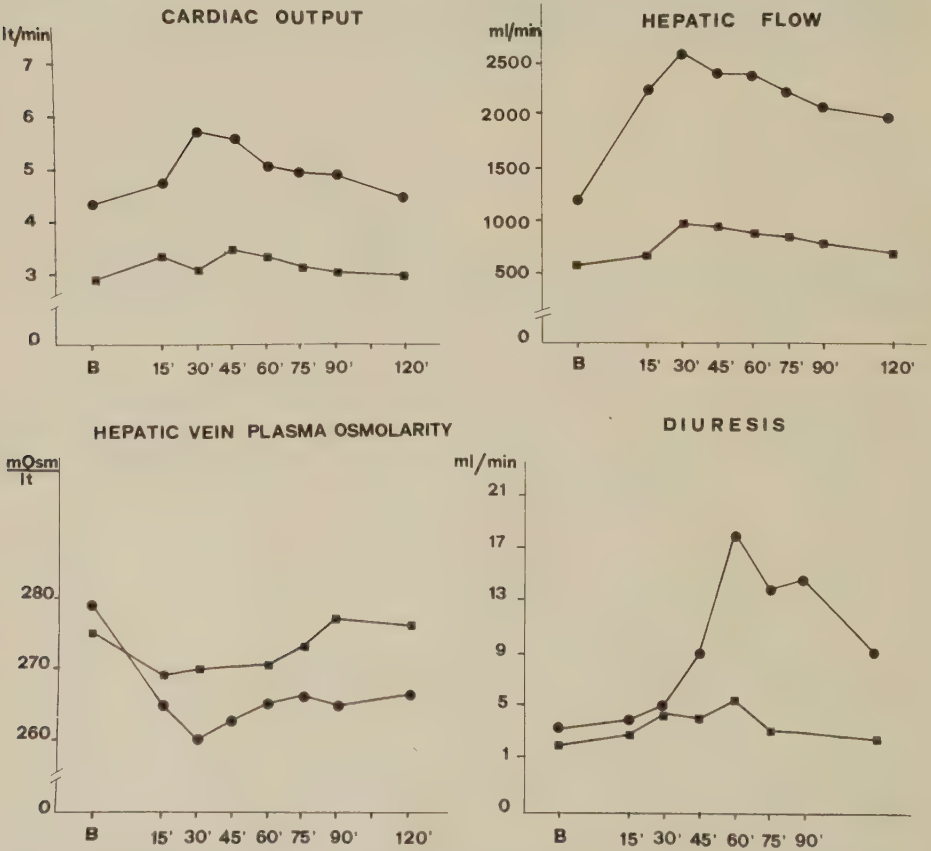


FIG. 11. Effects of water loading on different parameters measured in normal subjects (●—●) and in patients with CHF (■—■). Note that, plasma osmolality in blood collected from hepatic vein, and hepatic flow do not change in patients with CHF, as opposed to normals.

of collecting ducts, independently from the action of ADH, there is a backleak of hypotonic pre-urine in the hypertonic medullary space.

In patients with CHF, aldosterone plasma concentration during water loading remain almost unchanged (Fig. 8) (30-33).

Alteration in renal excretion of sodium chloride in CHF

Similarly to water retention, also sodium chloride retention in CHF is mostly due to an altered haemodynamic response to salt loading, which reflects on the renal haemodynamic and on the reabsorption by the proximal tubule. Isotonic and hypertonic salt loading affect similarly water and salt balance. Actually, hypertonic salt ingestion causes plasma hyperosmolarity that, in turn, induces the passage of fluids from interstitial and endocellular spaces toward the plasmatic space. This produces thirst and stimulates water assumption. Thus, hypertonic plasma becomes rapidly isotonic, with the final result of an increased plasma volume. In normal subjects the isotonic or hypertonic salt loading with meals, triggers immediately two compensatory mechanisms, leading to the reduction of water reabsorption at the level of the proximal tubule: 1) increase in cardiac output and in renal blood flow due to the increase in blood volume (a direct effect of an isotonic loading and an indirect effect of a hypertonic loading mediated by the transfer of fluid from the interstitial to the plasma space) (Fig. 12); 2) reduction of the plasma colloid osmotic pressure, due to hemodilution.

These two events, by the same mechanisms above described for water loading, induce a reduction of the net reabsorption at the level of the proximal tubule and an increase in sodium chloride delivery to the distal segment of the nephron. The increase in sodium urinary output is accompanied, either by a rise or by a decrease in urinary volume, depending on ADH secretion. Following an isotonic salt loading, ADH plasma concentration usually is reduced, probably as a consequence of the reduced PRA due to the increased renal blood flow (Fig. 13); therefore, the increased urinary sodium excretion is accompanied by an elevated urinary output. In contrast, following hypertonic salt loading, ADH secretion is stimulated by two stimuli, acting in opposite directions; one of them is represented by the elevated plasma osmotic pressure, that tends to increase ADH secretion, through osmotic receptors stimulation; the other is the rise in renal blood flow and the decrease in PRA, which tends to reduce ADH secretion. The increase in sodium excretion is accompanied by a fall or an increase in urine output depending on the prevalence of one stimulus or the other (8, 34). In any instance, aldosterone concentration falls, probably as a result of the increased renal blood flow and the consequent reduction in PRA, and this further enhances sodium excretion (35). Among factors determining the increased sodium excretion

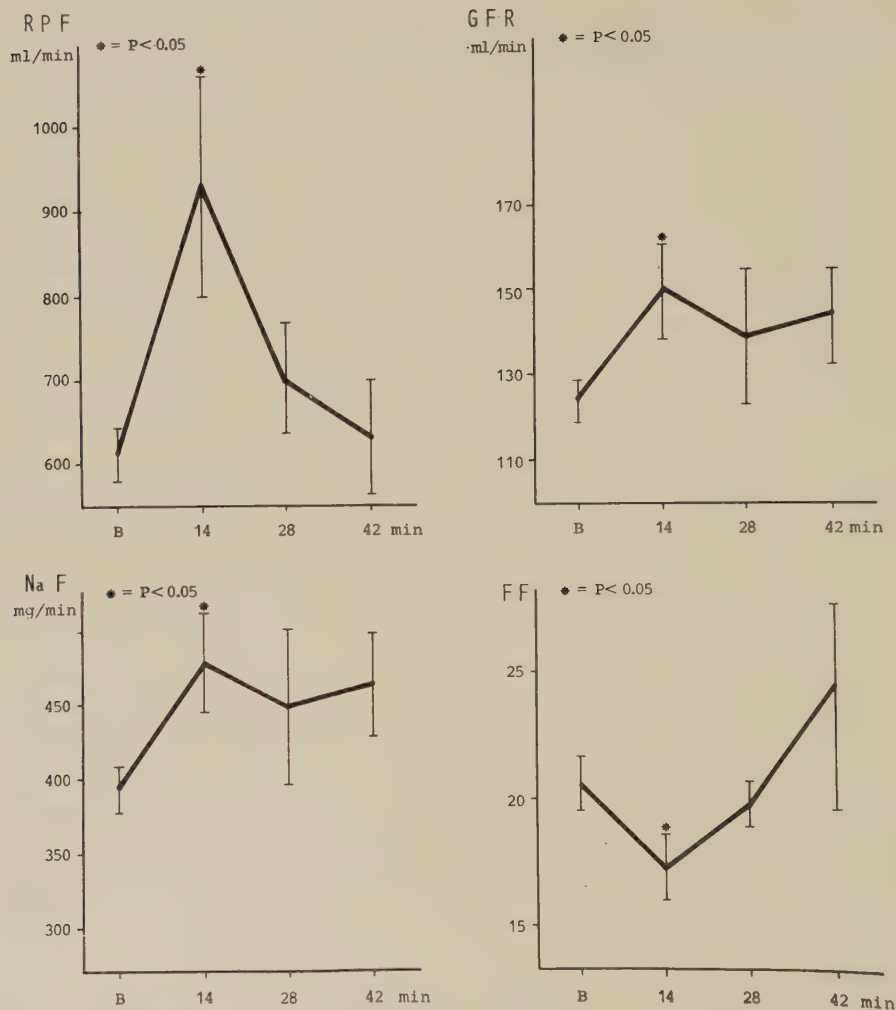


FIG. 12. Plasma renal flow (RPF), glomerular filtration rate (GFR), Na excretion (NaF) and filtration fraction (FF) in normal subjects before and after water loading added with 20 g NaCl. Hypertonic loading elevates plasma volume and therefore, RPF, GFR and NaF increase, while FF is reduced.

following salt loading, the most noteworthy is certainly the rise in renal blood flow. Renal vasodilation by itself is capable to increase sodium excretion, even when GFR does not change; however, the primary role played by the increase in renal blood flow in determining the enhancement of sodium excretion is demonstrated by experimental evidence; in a dog with a kidney perfused at constant flow, an intravenous loading with saline (500 ml over a period of 90 min) produced

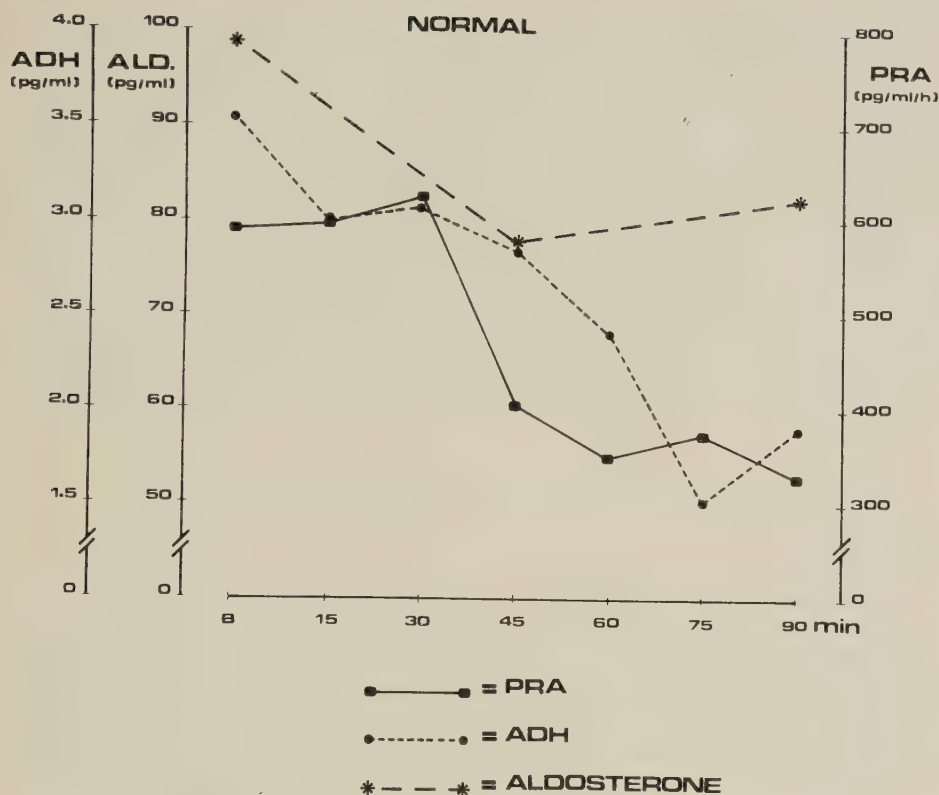


FIG. 13. Changes induced by saline load in isotonic solution on plasma renin activity (PRA), aldosterone (ALD) and antidiuretic hormone (ADH) in normal subject. The increase in renal plasma flow induces a reduction in PRA and ADH concentrations.

the increase in sodium and urine output only by the intact kidney, in which an increased RPF and GFR are also observed, while urine and sodium excretion of the perfused kidney, remain unchanged (Fig. 14) (36, 37). In CHF, isotonic and hypertonic salt loading are not accompanied by an increase in cardiac output and renal blood flow, which in the more severe cases can be also reduced. Since cardiac output and renal blood flow do not increase, or are even reduced, the net reabsorption at the level of the proximal tubule fails to be reduced, or is even increased: therefore, delivery to the distal segment of the nephron remains unchanged, or is reduced. When salt loading is isotonic and the reduction of renal blood flow is small, ADH and aldosterone plasma concentration do not change significantly and the amount of sodium excretion will depend on the amount of sodium that will reach the distal segment of the nephron and on the ADH and aldosterone plasma concentration present when salt loading is per-

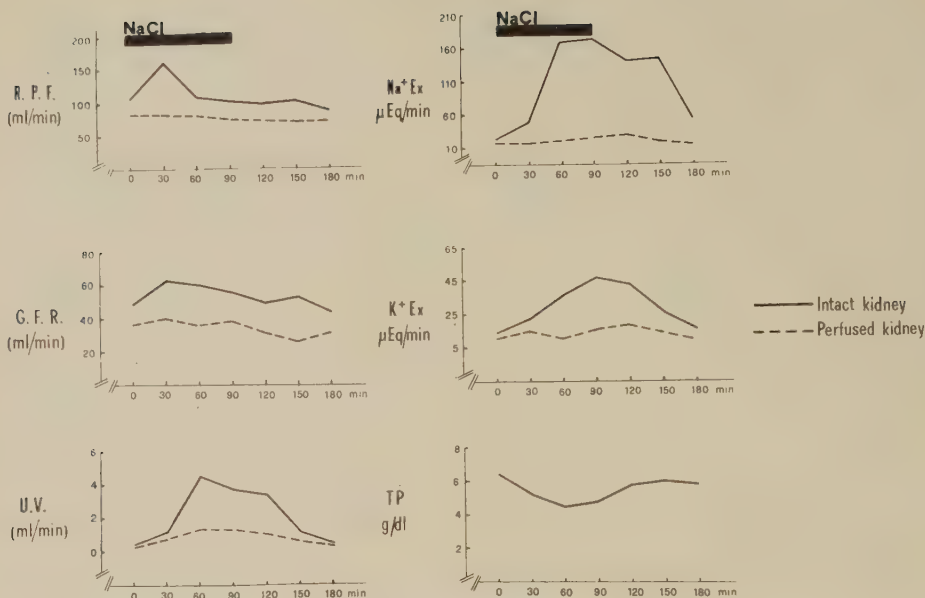


FIG. 14. Effects of 90 minutes isotonic NaCl solution infusion in a dog with one kidney perfused at constant flow with its own blood. Note that while in the intact kidney there is an increase in RPF, urinary volume, sodium and potassium excretion, such changes do not take place in the perfused kidney. RPF: Renal plasma flow; GFR: glomerular filtration rate; UV: urinary volume; NaEx: sodium urinary excretion; KEx: potassium excretion; TP: total plasma protein concentration.

med. Elevated levels of both hormones can be observed when the reduction in blood flow is so large that renin release is activated. With a hypertonic salt loading, ADH plasma concentration rises, as a consequence of the stimulation of osmotic receptors, this leading to a further reduction in urinary output; aldosterone blood levels either remain unchanged or are increased, for a critical reduction in renal blood flow that induces the activation of the renin-angiotensin-aldosterone system.

In conclusion, in CHF the main pathogenetic factor responsible for water and salt retention is haemodynamic and is represented by a pump failure of the impaired ventricle, responding with a depressed Frank-Maestrini-Starling curve to an increased venous return. This abnormal response by the failing ventricle is responsible for the venous and atrial congestion proximal to the ventricle itself, and also inhibits the sudden increase in cardiac output and, thus, in RPF, necessary for the fast restoration of osmotic, water, and salt balance. CHF takes place and becomes more severe gradually, following repetitive acute episodes of water and salt retention, due to the abnormal response of the pump function

to volume loading, when food and drinks are assumed. The hemodynamic disturbance mainly affects the absorption by the proximal tubule, while aldosterone and ADH play only a secondary role.

Hyperaldosteronism is not frequently found in CHF, being always absent during the initial period of the disease. Therefore, it plays only a secondary role in the pathogenesis of water and salt retention. Hyperaldosteronism in CHF may be caused by: 1) the reduction of hepatic clearance of the hormone, either for a fall in hepatic blood flow or for reduced hepatic extraction (38); 2) low-sodium diet and/or diuretic treatment; 3) increased sensitivity of renal tubules to the hormone; 4) failure of the mechanisms regulating hormone secretion. The same mechanisms regulating aldosterone secretion, probably, also affect ADH, its concentration rising with the increase of PRA (Fig. 15).

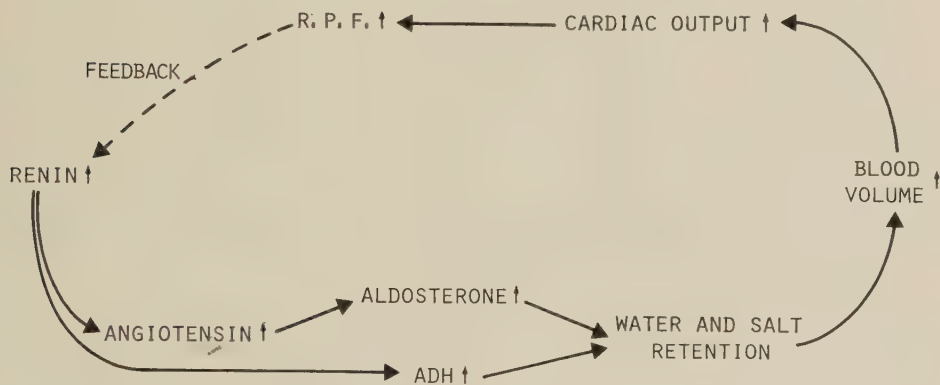


FIG. 15. Scheme of the renin-angiotensin-aldosterone system. When PRA increases, aldosterone and ADH concentration also rises, this inducing an increase in PRF, which in turns, reduces PRA.

When renal blood flow increases for the augmentation of blood volume, PRA falls, and this induces aldosterone and ADH release. The participation of ADH to the renin-angiotensin-aldosterone system is also shown by the similar changes observed in aldosterone and ADH concentration induced by the change in posture (Fig. 16). The presence of ADH in this system could also justify the so called "escape" phenomenon observed in the normal subject, but not in patients with CHF, during loading with exogenous aldosterone: in normal subjects, the increase in blood volume due to the water and salt retention induces a rise in venous return to the heart, in cardiac output and in renal blood flow; therefore, through the reduction in PRA, ADH concentration also falls, and an increase

in urine output takes place, that balances the sodium-retaining effect of aldosterone. In patients with CHF, in contrast, aldosterone, either endogenous or exogenous, though inducing an increase in blood volume, does not induce rise in cardiac output and renal blood flow and, thereby, does not affect ADH. As a consequence, an increased urine output is not observed, and water and

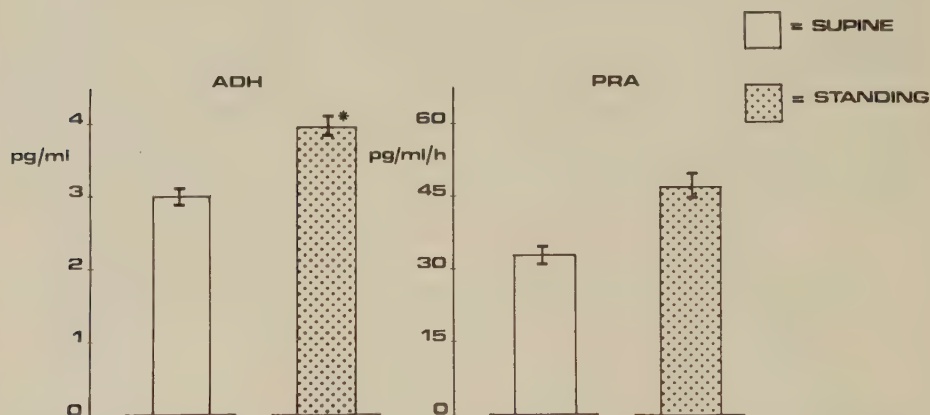


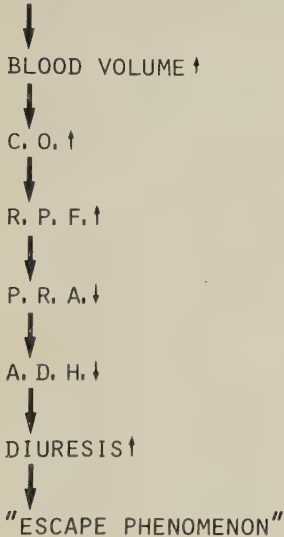
FIG. 16. Effects of change in posture (from supine to upright position) on PRA, plasma osmolarity (Osm), ADH and Aldosterone (ALD) plasma concentration, in normal subjects (solid columns) and in patients with CHF (shaded columns).

salt retention is more severe (Fig. 17). It has to be underlined that water and salt retention appears sooner and is more severe in right ventricular failure, than in left ventricular failure. It seems likely that in chronic left ventricular failure, for a certain period, the Henry-Gauer reflex may play a role- i.e. the inhibition of ADH secretion induced by the stretching of the left atrial wall, that, through the increase of urine output may reduce blood volume within physiologic limits. It is also likely that nicturia is induced by similar factors. When a patient with left ventricular failure develops also right ventricular failure, water and salt retention becomes more severe.

In the most advanced phase of CHF, water and salt retention is worsened by anatomic and functional changes intervening not only in those organs specially interested in maintaining water and salt balance (liver, kidney etc), but also in the cells of several tissues; thereby, the concentration and composition of intracellular electrolytes, and those functions that regulate osmolarity balance, are affected. Through these changes, the last phase of water and salt retention is reached, that can be defined as the phase of diffuse endocellular metabolic changes. Sometimes, these alterations are induced, at least partially, by medical mistreat-

NORMAL SUBJECTS

ALDOSTERONE ADMINISTRATION

PATIENTS IN HEART FAILURE

ALDOSTERONE ADMINISTRATION

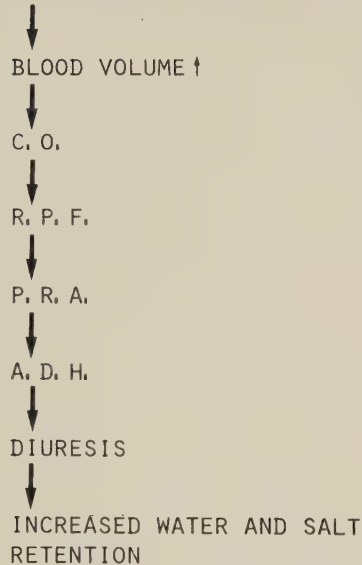


FIG. 17. Scheme of mechanisms of Aldosterone escape phenomenon in normal subjects and in patients with CHF.

ment (incongruous diuretic therapy); in most instances, at this step, CHF becomes refractory to any treatment.

Two of the pathophysiologic aspects of this latter phase of CHF have been thoroughly studied: the loss of potassium and hyponatremia.

Loss of potassium in CHF

It has been previously shown that in advanced CHF, there is a significant potassium depletion (40-42). This can be ascribed to three different causes: a) dietary deficiency that can be corrected by potassium administration; b) depletion induced by hypoxia, by acidosis and mostly by diuretics, that cannot be corrected by the simple potassium loading; c) pseudo-depletion, due to the loss of muscular tissue. In true potassium depletion, the place of potassium inside the cell is taken by sodium and hydrogen (2 Na^+ and 1 H^+ for 3 K^+), with the result of an increased intracellular sodium concentration, an intracellular acidosis and an extracellular alkalosis. For the reduced intracellular potassium concentration, the transmembrane potential is also reduced, this

implying a reduced conduction velocity of the stimulus and the development of late post-potentials. Both these factors are capable to generate severe arrhythmias (ectopic rhythms, ventricular fibrillation) (43, 44). The correction of intracellular deficiency cannot be achieved by potassium administration alone. Only by long-term administration of anti-aldosterone agents it is possible in the long run to restore the normal intracellular potassium concentration. In the pseudo-depletion, intracellular potassium concentration remains unmodified, since potassium loss is accompanied by simultaneous water loss and, thus, intracellular osmotic pressure remains unchanged and sodium and hydrogen do not penetrate into the cell. The amount of potassium lost with muscle dystrophy, can be restored only by the improvement of muscular trophism.

Hyponatremia in CHF

Hyponatremia is never observed during the initial period of CHF, while it generally heralds the eventual evolution of it. When hyponatremia is observed, it is a consequence of a quantity of total body water in excess as compared to sodium, although the absolute amount of sodium is increased. There are other diseases where hyponatremia is accompanied by a normal total body sodium content (hypersecretion of ADH in some brain tumors, in some cases of stroke, mixedema, infectious diseases, metabolic derangements; or hypersecretion of ADH-like substances as in some lung, prostatic or pancreas tumors); furthermore, in some instances, sodium body content can be also reduced (burns, diarrhea, vomiting, adrenal insufficiency, diuretic abuse, kidney disorders with electrolytes loss).

The pathogenetic mechanisms responsible for hyponatremia in CHF are probably the following: 1) due to the hemodynamic impairment, there is an increase in reabsorption at the level of the proximal tubule, this leading to an insufficient glomerular filtrate supply to the ascending part of the Henle's loop; 2) excess of ADH; 3) alteration in the electrolyte separation mechanisms in the ascending part of the Henle's loop, due to chloride pump damage induced by incongruous use of diuretics, such as furosemide, ethacrynic acid, thiazides: in these patients with damage chloride pump, the selective water excretion following water loading is impaired and, therefore, a water intoxication can develop; 4) alteration of the mechanisms regulating cellular osmotic balance.

Hyponatremia induces the passage of water into the cell, till the osmotic balance is restored between intracellular and extracellular fluids. Therefore, when tubular reabsorption of water and sodium increases to a similar extent, they maintain in the extracellular fluids the same ratio as when they are absorbed by gastro-intestinal tract; this induces hyponatremia even though the total body content is increased.

During the more advanced phase of CHF, hyponatremia may be the consequence of a severe impairment of cellular mechanisms regulating osmolarity balance, this representing the so-called "sick cell syndrome".

It is generally accepted that a derangement of intracellular metabolism may induce a fall in intracellular osmolarity, which, in turn induces a reduction in extracellular fluid osmolarity. It has been suggested that responsible for this derangement is the loss of intracellular phosphate esters or electrolytes.

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Mineral and water metabolism in renal diseases

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Water and mineral balance in patients with advanced renal failure may be maintained by the surviving nephrons only via an increase of the excretory contribution per residual nephron. Dietary reduction of water and solutes may contribute to the maintenance of the water and mineral metabolism but it is of limited value.

Adaptive processes in advanced renal failure can be realized by changes in single nephron GFR (SNGFR) and the rate of proximal and/or distal reabsorption and/or secretion of the particular substances usually excreted in urine. Therefore three forms of adaptations may occur in residual nephrons of advanced chronic renal insufficiency (5).

The first form concerns substances which are excreted by the kidney only by glomerular filtration. With reduction in GFR the serum concentration of these substances will rise. In consequence the filtered load (which is the product of GFR and serum concentration) of these substances per functioning nephron will increase. The only way to increase the excretion rate of these substances by the surviving nephrons is via an elevation of these substances in blood serum. This form of adaptation is termed *no regulation*.

The second form of adaptation, termed "regulation with limitation", concerns substances excreted by filtration and/or secretion in the tubules. Inorganic phosphates are examples of them. This type of adaptation is depended upon the reabsorptive and/or secretory capacity of the tubular epithelium of residual nephrons.

Finally the third type of adaptation (termed "complete regulation") is characterized by the maintenance of a normal serum level of a solute even at extremely low GFR (up to 2 ml/min.). Sodium, potassium and magnesium are falling into this class of solutes.

Functional characteristics of the surviving nephrons

It is now generally accepted that surviving nephrons in patients with advanced renal failure are undergoing several adaptations. In consequence of these adaptations one surviving nephron may accomplish the excretory work of even more

than 30-40 normal nephrons. The function of the residual nephrons is highly organized and responsive to the homeostatic needs of the patient despite profound changes in the architectural character of the diseased kidney. In human pathology homeostatic needs of patients with chronic renal failure are more often met by an adaptive inhibition of reabsorption or/and increase of secretion of a given solute by the tubules than by an increase in SNGFR.

Sodium excretion in advanced renal failure

It is a well known fact, that sodium excretion by diseased kidneys is precisely adapted in accordance with the need for the maintenance of external sodium balance. With progressing nephron loss fractional sodium excretion increases from a normal value of 0.5-2% up to 30% in order to maintain external sodium balance (11). In uremic patients increased fractional sodium excretion is caused by a decreased fractional reabsorption of this solute in the proximal tubule (which is only partially dependent upon the increased solute load per residual nephron) and by inhibition of sodium reabsorption in the distal tubule (9).

Among "natriuretic forces" probably involved in the mechanism of increased fractional sodium excretion an increase in SNGFR (15), a natriuretic factor (3, 8), redistribution of blood flow between superficial and deep nephrons and physical factors (increased osmotic load per nephron, increase of interstitial pressure etc.) are usually mentioned. The role of the renin-angiotensin-aldosterone system (10, 18) as well as of prostaglandins (20) and kinins in the process of increased fractional sodium excretion in advanced renal failure remains to be elucidated.

Despite an enormous increase of fractional sodium excretion per residual nephron the capacity for adaption necessary for the maintenance of sodium balance is not unlimited. The upper limit for sodium tolerance is dependent upon the number of residual nephrons, the pathology of renal disease and the effectiveness of adaptive natriuretic forces. The lower limit, which is often termed "obligatory excretion" of sodium in chronic renal disease, is a consequence of adaptive "natriuretic forces" and may range from 10 mmol/day or even less to more than two hundred mmol/day. Usually this value varies from 30 to 70 mmol/day at a GFR of 5 ml/min. Massive salt loss in urine in patients with chronic renal failure is usually termed as "salt losing". In the past this "salt-losing" was regarded as irreversible. It is now recognized as "reversible" and regarded as a consequence of adaptive "natriuretic forces". By slow and step-wise reduction of dietary sodium intake sodium losing can be suppressed to very low levels (less than 10 mmol/day) (6).

Potassium excretion in advanced renal failure

In normal persons about 10 per cent or less of the filtered potassium load is excreted. Most of the urinary potassium is derived from tubular secretion and not from glomerular filtration. In advanced loss of functioning nephrons (at a serum creatinine level of 10 mg/100 ml or higher) urinary excretion of potassium may exceed glomerular potassium load. The adaptive increase of potassium excretion per residual nephron relies predominantly on enhanced tubular secretion and not on inhibition of tubular reabsorption of filtered potassium (19). Normal aldosterone levels are indispensable for the maintenance of a normal serum potassium level in advanced renal failure (19).

An increase of non-aldosterone mediated tubular sodium-potassium activated adenosine triphosphatase (Na-K-ATPase) (13) and augmented delivery of sodium and water to the collecting duct (caused by the natriuretic hormone) are supposed to be responsible for the increased potassium excretion by residual nephrons.

Increased loss of potassium in stool is supposed to participate in the maintenance of potassium equilibrium in advanced nephron loss (2).

Increased cellular uptake of potassium as an adaptive mechanism of potassium tolerance in chronic renal failure was firmly excluded.

Phosphate excretion in chronic renal failure

In chronic renal failure external phosphorus balance and normal inorganic phosphate (Pi) level are maintained until the GFR falls to 25% of normal. Further decrement of GFR is usually accompanied by an increase of serum phosphate level and urinary fractional excretion, evoked by a decrease in tubular phosphate reabsorption.

Studies performed by Slatopolsky *et al.* (14) have emphasized the role of parathyroid hormone (PTH) in the adaptive mechanism of increased phosphate excretion per residual nephron. In dogs, with reduced GFR development of secondary hyperparathyroidism may be prevented by proportional reduction of phosphate intake (14). Studies of the last years seem to prove that PTH is not the dominant controlling factor for tubular reabsorption of inorganic phosphate and that a PTH-independent mechanism may be involved in the maintenance of phosphorus balance. It was demonstrated that renal phosphate excretion is mainly dependent upon the dietary phosphate intake (7, 16).

Water excretion in chronic renal failure

Maintenance of water homeostasis in advanced renal failure is realized through an increased fractional excretion of filtered water by residual nephrons (17). The

maximally obtainable water excretion cannot exceed $1/3$ of the GFR. This fact is of great therapeutic importance. In order to excrete 450 mmol of solutes (this is the minimal quantity of solutes usually supplied by a normal diet) in an isotonic (-300 mmol/kg H_2O) urine i.e. in a volume of 1,5 liters at least 4500 ml of glomerular filtrate per day must be elaborated. This volume of glomerular filtrate corresponds to a GFR of 3 ml/min. This example visualizes the nonsense of prescribing large quantities of fluids in order to force diuresis in patients with advanced renal failure.

Free water clearance (CH_2O) per residual nephron is relatively well maintained even in advanced uremia. Despite this "free water" usually contributes very little to the osmolarity of urine rendering it slightly hypotonic.

Advanced renal failure is always accompanied by a concentrating defect. The cause of this defect seems to be multifactorial (increased solute load per residual nephron with subsequent osmotic diuresis, resistance of collecting ducts to vasopressin, decreased tubular sodium reabsorption impairing the elaboration of a hypertonic medulla, disruption of the important anatomic relationship between tubules, interstitium and blood vessels - 1, 17).

Are humoral components of adaptive mechanisms conditioning water-mineral homeostasis in advanced renal failure, uremic toxins?

As already pointed out increased serum PTH is supposed to play an important role in the adaptive mechanism of increased phosphate excretion per residual nephron in advanced renal failure. In last years evidence was collected sufficient to indict this hormone as a uremic toxin, responsible, at least in part, for neurologic abnormalities, anemia, pruritus, bone necrosis, osteitis fibrosa, uremic hypertriglyceridemia and impotence (12). The assumption, that some abnormalities of the uremic syndrome may be consequences of extrarenal effects of humoral components of adaptations in nephron function, was the basis of the so-called "trade-off" hypothesis (4). According to this hypothesis not only extrarenal effect of PTH but also of the natriuretic hormone were related to some uremic abnormalities (4, 5). If the hypothesis is right prevention of adaptive mechanisms by decreasing excretory requirements per residual nephron should prevent the "trade-off" phenomena.

Summarizing it can be stated that experimental and clinical studies of the last ten years have very much contributed to our understanding of adaptive mechanisms conditioning maintenance of the water-electrolyte balance even in advanced renal failure and to the conservative therapy of uremic patients. In the coming years nephrologists will be confronted more with the question: "Are adaptive mechanisms in advanced renal failure responsible for any uremic

abnormalities?'. The answer to this question will undoubtedly influence the therapy of chronic renal failure.

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Mechanisms of diarrhoea

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The most obvious manifestation of disturbances of salt and water metabolism in the gut is diarrhoea and this chapter will focus on the mechanisms by which such disturbances may arise.

Large amounts of salt and water are handled daily in the gut. Some 7 litres of fluid enter the duodenum each day, made up of food and secretions from stomach, pancreas, biliary tract and saliva. Of this, the vast majority is absorbed in the small bowel, leaving only a litre or so to pass on into the colon. The colon wrings its contents fairly dry so that only about 100 ml is allowed to escape in the stools each day. It has been possible to elucidate, at least in part, the mechanisms by which this salt and water are absorbed and this information will be used here to illustrate the ways in which malabsorption may occur and diarrhoea results.

Such disturbances may occur by several possible mechanisms. Firstly, *osmotic diarrhoea*, produced by unabsorbed solutes in the lumen of the gut drawing water into the lumen, by osmotic pressure and retaining it there. Secondly, changes in the *permeability* of the gut to salt and water is likely to have a profound influence. Thirdly, the *inhibition of those active processes*, requiring energy, for the absorption of salt, processes which become increasingly important in the ileum and colon, may result in malabsorption of salt with production of diarrhoea. Finally, the stimulation of some process by which the intestine is made to *secrete* salt and water could result in diarrhoea. Motility disturbances will not be discussed.

Dealing with each mechanism in turn, the commonest type of osmotic diarrhoea is probably that due to lactose intolerance. A deficiency of the enzyme lactase, normally present on the brush border membrane, results in a failure of digestion and hence absorption of this disaccharide. It remains in the lumen, keeping water with it in an isosmotic solution, that is at about the same osmotic pressure as that on the other side of the mucosa in interstitial fluid and plasma. Furthermore, as the lactose reaches the colon, it is metabolised by colonic bacteria to an increased number of smaller solutes which draw yet more water into the lumen to maintain equal osmotic pressures on each side of the mucosa.

In coeliac disease the bowel disturbance is usually considered as giving rise to steatorrhoea, but it is clear that many other materials are incompletely absorbed including water soluble substances. These substances certainly add an osmotic load to luminal contents which must encourage the diarrhoea. Such solutes will include unabsorbed carbohydrate and protein.

It is clear that the case with which salt and water permeate the intestinal mucosa is markedly impaired in coeliac disease. This may be due to the reduced surface area of the intestine but, since most salt and water passes across the jejunal mucosa between the epithelial cells rather than through them, and since it is known that the mucosal architecture is grossly disturbed in coeliac disease, it seems likely that the decreased mucosal permeability in this disease is due to the distorted lateral spaces between epithelial cells. It has also been shown that the permeability of both small and large bowel in Crohn's disease, and the large bowel in colitis, is changed and this factor may well enhance the liability to diarrhoea in these diseases.

It is clear that there are a number of specific active absorptive mechanisms for the transport of electrolytes across intestinal mucosa. It is not surprising, therefore, to find that diarrhoea may result from defects in these normal processes. In coeliac disease, it is likely that mucosal 'atrophy' will cause not only impairment of absorption of nutrients, but also of electrolytes in so far as they rely on specific active mechanisms. Such active processes for salt absorption achieve most significance in the ileum and colon and it is likely that only in those coeliac patients with disease severe enough to extend down into the ileum, that this defect will become obvious in diarrhoea.

There is only one defined congenital defect in intestinal electrolyte transport which has been described thus far, and that is "congenital chloride losing diarrhoea". A great rarity, it is characterised by severe watery diarrhoea from birth, a metabolic alkalosis and an excessive loss of chloride ions in the stools. The defect has been ascribed to the absence of the normal transporting enzyme for chloride in the ileal and colonic mucosa of these patients. Since chloride is probably absorbed in exchange for bicarbonate secreted, the chloride loss and metabolic alkalosis can be explained by the absence of this "exchange" process.

Some recent experimental work in Toronto has shown that in one form of viral gastroenteritis, the diarrhoea may be due, at least in part, to a specific enzyme defect in the small intestinal mucosa. It has been shown that in pigs with experimental viral enteritis, there are reduced levels of the enzyme—Na. K. ATP ase—which may be rate-limiting for sodium absorption in the mucosa. This may be due to structural alterations since the enzyme is present predominantly in the villi and these are stunted in this condition.

Recent attention has focussed on intestinal secretion as a mechanism for

diarrhoea. The main stimulus for this work has come from studies of the most severe sort of secretory process which has been recognised—namely that of Asiatic cholera. In this disease, the diarrhoea may reach 10 or 20 litres of fluid per day, mainly an isotonic bicarbonate-saline solution. It has been shown that this loss occurs predominantly from the jejunum and that, despite this enormous secretion of fluid, the jejunal mucosa looks virtually normal histologically. Furthermore, despite secreting fluid so rapidly, this mucosa is fully capable of absorbing glucose and amino acids normally too, and the enhanced absorption of salt and water which accompanies glucose absorption occurs normal too. This has had two important implications. Firstly, the extremely important practical implication for therapy with the use of oral glucose-electrolyte solutions. This has made an enormous difference to the management of these patients, since it is possible to rehydrate many such patients with oral therapy without recourse to expensive and scarce intravenous fluids. Secondly, it indicates that the abnormality in this condition is a very specific biochemical one which leaves mucosal structure and other functions intact. Further work has shown that it is the toxin of cholera which is responsible for switching on secretion which can occur after even a very brief 30 second exposure in experimental conditions. After removal of the toxin, by washing, there is a short delay of about $\frac{1}{2}$ to 1 hour before secretion starts. Once switched on, however, it continues for 24 hours or more and probably until those epithelial cells which have been switched on have been shed at the end of their life cycle. It is clear therefore that some specific biochemical secretory mechanism has been triggered and it has now been recognised that this intracellular mechanism involves the second messenger, cyclic adenosine monophosphate (cyclic AMP).

Cyclic AMP is produced from ATP under the influence of the membrane bound enzyme adenyl cyclase. Cyclic AMP is broken down in the cell by phosphodiesterases to AMP. Now adenyl cyclase is activated by a variety of stimuli—predominantly hormones, and specificity of action is determined by specificity of receptors for the hormones. It has now been shown that adenyl cyclase activity in intestinal mucosa is stimulated by cholera toxin and cyclic AMP levels increase as a result. Furthermore, exogenously administered cyclic AMP stimulates the same sort of intestinal secretion, in experimental conditions, as does cholera toxin. So the theory has developed that cholera toxin acts by stimulating cyclic AMP production in the mucosa which, in turn, evokes the secretion.

The theory has gone further than that because it has been proposed that this, normally present, secretory system may be stimulated by other types of diarrhoea producing infective agents. Some evidence in favour of this concept has been derived for *E. coli*, heat labile toxin, *C. welchii*, staphylococcal food poisoning, shigella and Klebsiella. The evidence is still shaky for some of these

but this should not detract from this nice concept of similar intracellular mechanisms for different aetiological types of diarrhoea.

This mechanism may also be involved in non-infective diarrhoeas and it has, for instance, been shown that certain long chain fatty acids and deconjugated bile salts can each stimulate cyclic AMP production and intestinal secretion—at least in the colon. Such may be the case, for example, after ileal resection when bile salts spill over, in excess, into the colon. A similar mechanism may be involved in adding to the diarrhoea in patients with steatorrhoea since hydroxy fatty acids also stimulate colonic adenyl cyclase.

This process may be involved in some of the rarer forms of hormonally induced diarrhoeas. In the “Verner Morrison” syndrome of pancreatic cholera, which is associated with a tumour of the Islets of Langerhans, it has been clearly shown that the tumours almost always secrete large amounts of vaso-active intestinal peptide (V.I.P.) into the circulation. V.I.P. has also been shown to stimulate mucosal cyclic AMP production and intestinal secretion and is probably responsible for the diarrhoea.

Another class of compound may act by similar mechanism. Prostaglandins, often produced in excess in patients with medullary carcinoma of the thyroid, have been shown to stimulate cyclic AMP and may be responsible for the diarrhoea found in this disease.

Clearly, this single mechanism is unlikely to be the basis for all forms of diarrhoea and it is likely that yet other mechanisms will be discovered for other forms of diarrhoea. Thus, serotonin, liberated in excess in patients with carcinoid syndrome, causes diarrhoea by stimulating secretion by non-cAMP mediated means and it is likely that the heat stable toxin of *E. coli* provokes diarrhoea by mechanisms not involving cAMP. But the discovery of these processes has opened new insights into possible therapeutic approaches for these diseases. Thus each of the steps in the biochemical chain of events leading to secretion has been subject to experimental attack by chemical means. As yet, few of these have shown therapeutic promise although recently chlorpromazine, an adenyl cyclase inhibitor, was shown to reduce the severity of diarrhoea in a group of patients with cholera.

This sort of biochemical manipulation is leading us into an exciting era which ultimately may lead to specific remedies for specific types of diarrhoeal disease.

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Diuretics and problems of water and salt metabolism

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The Action of Diuretics

Diuretics are drugs that increase the urinary excretion of salt and water. They generally act in the kidneys by inhibiting electrolyte transport in the renal tubular epithelial cells, so that a greater fraction of sodium and water is excreted in the urine. The effect on water and electrolyte excretion of a diuretic depends on the site of action in the nephron (Burg 1976).

Acetazolamide and other carbonic anhydrase (c.a.) inhibitors act mainly in the proximal tubulus by inhibiting bicarbonate reabsorption, but has probably also an effect in the distal tubulus. Thiazides and thiazide-like drugs, such as chlorthalidone, mefruside, and metolazone exert their main action in the first part of the distal tubulus, i.e. the cortical diluting site, where they inhibit sodium chloride reabsorption. They also exert a weak c.a. inhibiting effect to a varying degree. Furosemide, bumetanide, and ethacrynic acid, the so-called loop diuretics, exert their main effect in the ascending limb of Henle's loop, where they inhibit chloride transport. They are able to increase sodium and chloride excretion more than the thiazides and are active in severe renal failure, where thiazides have no effect. Furosemide and bumetanide are weak c.a. inhibitors, but ethacrynic acid lacks such an effect. Mercurial diuretics presumably also have their action on chloride transport in the ascending loop of Henle. At last, there are diuretics, which act on the sodium reabsorption and potassium secretion in the distal tubulus and collecting duct. The spironolactones are steroid analogues of the mineralocorticoid hormones, which competitively inhibit the effect of aldosterone and other mineralocorticoids. Two other drugs, amiloride and triamterene, have a similar effect on the distal ion exchange mechanism but are effective in the absence of mineralocorticoids and seem to have a direct effect on transepithelial sodium transport. All these three drugs increase sodium excretion and decrease potassium excretion (potassium-sparing diuretics). These diuretics are relatively weak diuretic agents, when used alone. By combining them with more powerful diuretics, such as thiazides and loop diuretics, the sodium excretion is enhanced, while loss of potassium is prevented.

The effects of diuretics on sodium and potassium excretion are summarized in Table 1. It appears that all diuretics which act proximal to the distal potassium-secreting site (e.g. loop diuretics and thiazides) increase urinary potassium excretion due to increased delivery of sodium available for exchange with potassium; the ion exchange is stimulated by secondary aldosteronism.

TABLE 1
Effect of diuretics on sodium and potassium excretion

	Na ⁺	K ⁺
Thiazides, chlorthalidone, mefruside, metolazone	↑ ↑	↑ (↑)
Furosemide, bumetanide, etacrynic acid	↑ ↑ ↑	↑
Acetazolamide (c.a.-inhibitor)	↑	↑
Spironolactone, triamterene, amiloride	↑	↓

Indications for Diuretics

The main indications for therapeutic use of diuretics are different oedematous states (cardiac oedema, nephrotic syndrome, renal failure, liver cirrhosis, premenstrual oedema, local oedema) and hypertension. In addition, other more rare conditions, such as hypercalcaemia (loop diuretics), hypercalciuria (thiazides) and diabetes insipidus are treated with diuretics.

Some Adverse Effects of Diuretics

1. *Sodium depletion.* It is not surprising that strongly acting diuretic drugs may induce sodium depletion. This is not a side-effect in the strict sense, but rather a sign that the treating physician fails to appreciate the patient's condition before signs of sodium depletion develop. Treatment of marked cardiac or nephrotic oedema may require high doses of e.g. furosemide initially, especially if the patient has also reduced glomerular filtration. However, when the patient has reached the dry weight, the doses of diuretics should be modified as to keep the body weight constant in order not to further reduce the sodium content of the patient. Sodium depletion induced by diuretics gives rise to weight loss below "dry weight", lowered skin turgor, arterial hypotension with marked orthostatic reaction, and signs of haemoconcentration (high haematocrit and plasma protein). Some patients develop hyponatraemia.

Sodium depletion may be especially dangerous in patients with renal failure, where the drop in extracellular volume and blood pressure may give rise to further decrease in glomerular filtration with azotemia and signs of uraemic toxicity.

In patients with the nephrotic syndrome, which may have a contracted plasma volume due to hypoproteinaemia, too vigorous treatment with saluretic drugs may induce shock-like conditions, due to hypovolaemia. One way to avoid such a reaction is to give the patient colloid solutions (plasma, albumin, dextran) in order to mobilize sodium and water from the tissues and to restore the plasma volume.

2. *Dilutional hyponatraemia.* This condition may be a consequence of diuretic therapy (cf. Miller and Moses 1976) and may occur in spite of increased total body sodium content. Contributing factors may be reduction in glomerular filtration rate with enhanced reabsorption of sodium and water proximal to the diluting sites and direct inhibition of sodium chloride reabsorption in these segments, thereby limiting maximum free water generation. Concomitant potassium depletion and increased release of antidiuretic hormone may also be of importance. An appropriate therapeutic measure is water restriction. Potassium repletion may also return plasma sodium to normal in a few patients (Fichman *et al.*, 1971).

3. *Potassium depletion.* One of the most common findings in patients treated with thiazides or loop diuretics is hypokalaemia, associated with hypochloraemic alkalosis, which may be a sign of potassium depletion. Normal subjects given hydrochlorothiazide, 150 mg/d for one week, show a 6% decrease in muscle potassium (percutaneous needle biopsy) with an increase in intracellular sodium (Bergström and Hultman 1966). The degree of potassium loss appears to be dose-related and may be excessive in subjects who ingest diuretics surreptitiously: A 29-year old woman, who took large amounts of furosemide and chlorthalidone was found to have suffered a 26% decrease in whole body potassium and muscle potassium with a marked increase in intracellular sodium and the basic amino acids, ornithine, lysine, and arginine (Bergström *et al.*, 1976).

It has been a matter of controversy whether the slight to moderate hypokalaemia, which is generally observed during low-dose, long-term treatment with diuretics belonging to the thiazide group is a sign of potassium depletion or not.

We investigated patients with hypertension before and after treatment for 4 or 5 months with mefruside, 25 mg/d, and found no decrease in muscle potassium in spite of slight hypokalaemia (Bergström *et al.*, 1973, Bevegård *et al.*, 1977). Several studies of whole-body potassium confirm these results (cf. Kassirer and Harrington 1977).

Typical symptoms of potassium depletion are anorexia, vomiting, obstipation, polydipsia, and polyuria. In more severe cases, muscle paralysis and cardiac arrhythmia. The danger of potassium depletion in patients on digitalis should

be emphasized; the sensitivity to digitalis will be increased and the risk of intoxication enhanced.

Potassium supplementation should be given to patients who are actively treated for oedema (cardiac failure, nephrosis, liver insufficiency), especially if high dosage of diuretics are required. Patients on low maintenance dose probably do not require extra potassium, except in the elderly patient, where the potassium intake by food may be inadequate. When potassium is given, it should preferably be in the form of potassium chloride and as slow-release tablets to avoid side-effects from the gastrointestinal tract. An alternative to giving potassium supplementation is to combine thiazides or loop diuretics with a potassium-sparing diuretic, i.e. an aldosterone antagonist (spironolactone), triamterene or amiloride.

When we gave amiloride (15 mg/d) together with hydrochlorothiazide (150 mg/d) to normal subjects for one week, we were able to completely prevent the intracellular potassium loss induced by hydrochlorothiazide alone, as well as the increase in intracellular sodium concentration, demonstrating the efficiency of amiloride as potassium-sparing agent (Bergström and Fridén 1975). By combining hydrochlorothiazide with amiloride we could also prevent most of the side-effects of hydrochlorothiazide given alone (fatigue, nausea, vomiting, increased thirst), indicating that these side-effects were related to potassium depletion more than to hypovolaemia. When using potassium-sparing drugs, it must be remembered that they may induce hyperkalaemia in patients with reduced renal function. These drugs are therefore contraindicated in patients with renal failure.

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SYMPOSIA
SO-CALLED HEPATO-RENAL SYNDROME

The so-called hepatorenal syndrome: introductory remarks

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The term hepatorenal syndrome was introduced more than 4 years ago to define the frequent association between hepatobiliary disease and renal failure (Lichtman and Sohval 1937). Since that time more than 20 different clinical conditions in which this association occurs have been described (Baldus and Summerskill 1975). For this reason the term hepatorenal syndrome should be avoided since it is too imprecise and confusing. The most frequent and characteristic type of renal failure in cirrhosis is the syndrome of functional renal failure (Vesin, 1962), which is present in approximately 20% of patients with cirrhosis and ascites admitted to a General Hospital (Bosch 1973). Functional renal failure is characterized by a progressive azotemia and oliguria, a low urine sodium concentration, and a urine/plasma osmolality ratio over 1.1 (Rodés *et al.*, 1975). Many of these patients die and, on post-mortem examination, the kidneys are found to be morphologically normal. Most of the current Symposium is devoted to discuss this type of renal failure. However, it should be remarked that patients with cirrhosis and ascites may present other types of renal failure, and particularly, to stress that renal failure in cirrhosis is frequently iatrogenic.

The administration of nonsteroid anti-inflammatory drugs, which are potent inhibitors of prostaglandin synthesis, may induce the development of functional renal failure, which usually reverses if the responsible drug is early withdrawn (Arroyo *et al.*, 1978).

Another type of renal failure which frequently appears in cirrhosis with ascites is that induced by diuretics. The pathogenesis of diuretic-induced renal failure is related to the fact that ascites resorption from the peritoneal cavity is a rate limited phenomenon (Shear *et al.*, 1970). So, if diuresis is established over the ascites reabsorption rate, a contraction of the plasma volume occurs, and may give rise to a drop of the glomerular filtration rate and therefore to diuretic induced renal failure.

The two most important factors influencing ascites resorption are the degree of portal hypertension and the protein concentration of ascites (Bosch *et al.*, 1977). In a series of patients with cirrhosis and ascites submitted to diuretic

therapy, we found that most patients with diuretic-induced renal failure had a wedged hepatic pressure over 30 mm of Hg or an ascites protein over 20 gr/l. In our experience, diuretic-induced renal failure reversed on the interruption of the diuretic therapy.

Patients with decompensated cirrhosis are prone to develop infections by Gram negative bacteria and therefore, they are frequently treated with potentially nephrotoxic antibiotics. In our experience the incidence of antibiotic loading to acute tubular necrosis is very high in cirrhosis with ascites.

Recent investigations have shown that an infrequent nephropathy, the mesangio-capillary glomerulonephritis with IgA and complement deposits in the glomeruli may develop with an unusual high frequency in cirrhosis (Nochy *et al.*, 1976). These patients usually present renal failure, hematuria, proteinuria and arterial hypertension. Its pathogenesis is not clear but it has been suggested that it may be due to deposition of circulating immunocomplexes formed by the reaction of antibodies against antigens of intestinal origine.

So, patients with cirrhosis and ascites may develop several types of renal failure with different etiology, prognosis and pathogenesis. This Symposium has been organized in order to discuss some aspects of renal impairment in liver diseases.

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Renal involvement in chronic hepatitis

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Actually the hepatorenal syndrome is considered a pathological picture characterized by functional renal alterations occurring during chronic liver diseases (Conn, 1973; Sherlock, 1975).

These alterations consist in water and salt balance displacement, lowering of renal clearances (renal plasma flow: RPF and glomerular filtration rate: GFR) and altered perfusion of the kidney, leading to an ever worsening azotemia and a therapy-resistant water retention (Vesin, 1962; Baldus *et al.*, 1964; Epstein *et al.*, 1970; Sherlock, 1975).

Morfological findings are on the contrary modest and never sufficient to justify such a degree of renal impairment (Papper *et al.*, 1959; Vesin, 1962; Baldus and Summerskill, 1975).

Renal failure has been described mainly during cirrhosis; however, also in stages of chronic active hepatitis (CAH) without cirrhosis and portal hypertension, slight modifications of kidney function can be detected before the clinical signs of the typical hepatorenal syndrome, such as azotemia, are evident (Gentilini *et al.*, 1975, 1976).

In patients with cirrhosis, abnormally high levels of prostaglandins (PGE_1 and PG_2) in venous and more so arterial renal blood were also observed (Gentilini *et al.*, 1977).

The most important finding, however, seems to be the opening of intrarenal artero-venous shunts, detected in patients with cirrhosis (active cirrhosis: AC) and CAH by using $^{99\text{m}}\text{Tc}$ -microspheres of human albumin (30 μm diameter), injected into the renal artery (Gentilini *et al.*, 1975, 1976).

The aim of our research is to confirm in a wider number of cases the presence of these renal functional modifications and to compare them with intrarenal hemodynamics, plasma renin activity (PRA) and PGE_1 and PGE_2 levels in the renal blood both in patients with CAH and AC.

METHODS

95 patients, 47 males and 48 females, ranging from 18 to 69 years, with a mean age of 49 years, were studied: 54 had CAH and 41 AC; 17 cases were

HBsAg positive. 12 patients with chronic persistent hepatitis (CPH) were considered as controls.

11 subjects had no past or present renal or cardiovascular disease, no water retention, normal values of BUN, creatininemia, and bilirubinemia values below 2 mg/100 ml. They were given a standard diet with 2.5 g sodium chloride per day and no therapy during the observation period.

Blood gas analysis, urinary pH, bicarbonaturia, ammoniuria and titratable acidity were assessed before and after ammonium chloride loading test, in order to study renal tubular function.

RPF and GFR were tested by using sodium-p-aminohyppurate and sodium tiosulphate; in some cases ^{131}I -radiohyppaque was also used.

Selective renal arteriography was performed on 47 patients in order to study intrarenal hemodynamics. A perfusion kidney scan was also carried out on 26 of this group by injecting 1-2 mCi of the tracer ($^{99\text{m}}\text{Tc}$ -microspheres of human albumin of 30 m diameter, with a specific activity of 5 mCi/mg) into each renal artery, through the catheter already inserted for angiography.

Blood specimens for prostaglandins and renin determination were collected from both renal arteries and veins just before the tracer infusion. Prostaglandins were estimated with the RIA method (Clinical Assay Kit), according to Jaffe and Berhman (1973) and PRA by measuring generated angiotensin I (NEN Kit).

As regards to other methods and technical details we refer to a preceding work (Gentilini *et al.*, 1976).

RESULTS

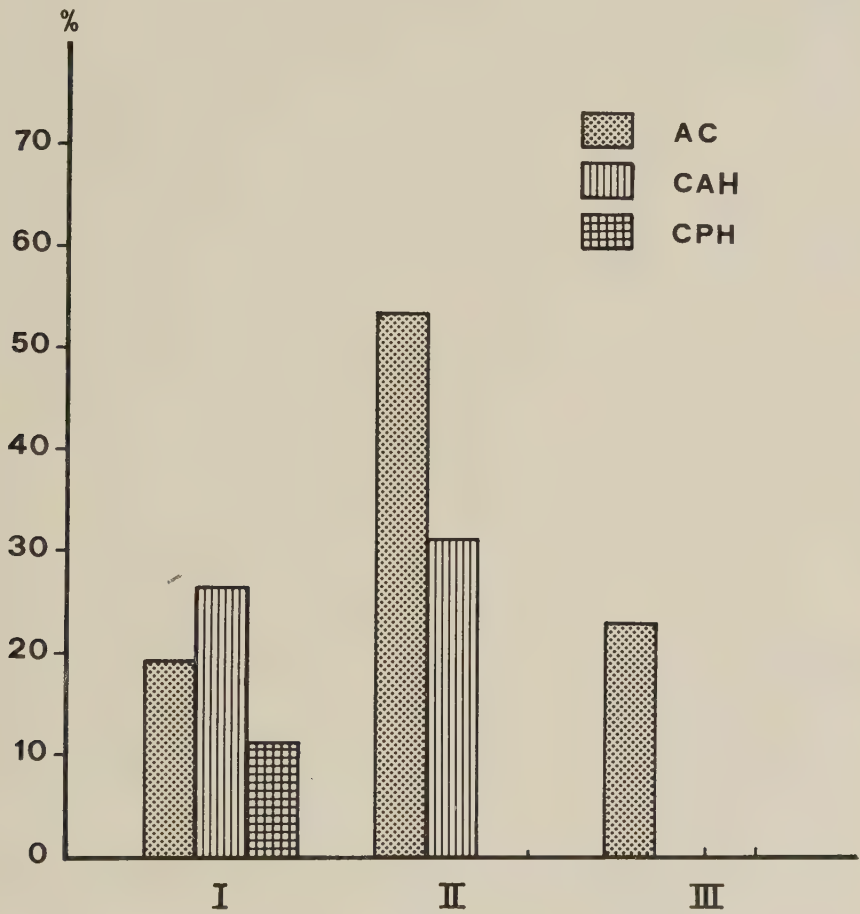
The main urine data are reported in Tables 1 and 2: bicarbonate mean values result altered mainly in cirrhosis; proximal tubular acidosis (RTA II) was found in 5/26 (19.2%) patients with AC, in 9/34 (26.5%) with CAH and only in 1/9 case with CPH; distal tubular acidosis (RTA I) was found in 7/13

TABLE 1

Mean values (\pm SEM) of some urinary parameters in patients with active cirrhosis (AC), chronic active hepatitis (CAH) and chronic persistent hepatitis (CPH)

	No. of cases	pH	bicarbonate n.v. = 0-5 mEq/l	ammonium n.v. = 11-27 mEq/24h	acidity titratable n.v. = 7-24 mEq/24h
AC	26	6.3 ± 0.1	11.6 ± 2.4	48.3 ± 6.8	30.9 ± 3.3
CAH	34	6.2 ± 0.1	7.8 ± 1.7	39.2 ± 4.6	32.2 ± 2.3
CPH	10	6.1 ± 0.1	7.4 ± 3.1	40.5 ± 5.4	33.4 ± 6.2

TABLE 2
Incidence of cases with renal tubular acidosis



I: renal tubular acidosis type 2 (proximal);
II: renal tubular acidosis type I (distal);
III: proximal and distal associated renal tubular acidosis.

(53.8%) patients with AC, in 5/16 (31.2%) with CAH and in none out of 4 cases with CPH; both types (RTA I + RTA II) were observed only in 3/13 patients (23%) with AC.

RPF and GFR (Table 3) were significantly decreased respectively in 20 (51%) and 17 (43%) patients with AC, in 20 (37%) and 17 (32%) with CAH; no significant alterations was observed in CPH.

Selective renal arteriography was considered abnormal when it showed an inhomogeneous and decreased distribution of the contrast medium in the kidney outer cortex, which appeared thinner and with irregular intrarenal arteries, thus suggesting cortex hypoperfusion.

TABLE 3

Mean values (\pm SEM) of renal plasma flow (RPF) and glomerular filtration rate (GFR) with indication of altered cases

	RPF				GFR		
	No. of cases	Means (ml/min)	No. altered	%	Means (ml/min)	No. altered	%
AC	39	425.9 \pm 32.7	20	51.0	96.9 \pm 6.0	17	43.0
CAH	53	467.1 \pm 28.9	20	37.0	99.8 \pm 5.5	17	32.0
CPH	12	601.7 \pm 47.3	0		125.8 \pm 13.1	0	

Similar findings were observed in 16/30 (53.3%) with AC and in 10/16 (62.2%) with CAH.

6/13 (46.2%) patients with AC and 4/22 (33.3%) with CAH had a scan in which an evident escape of the tracer was observed from the kidney towards the lung; this finding strongly indicates the presence of intrarenal artero-venous shunts.

As these methods may cause discomfort to the patient and had given negative results in 15 patients with suspected renal involvement, only one case of CPH underwent arteriography and scan, which showed no alteration.

Renal arterial and venous PGE₁ and PGE₂ mean values (\pm SEM) resulted respectively 433.2 \pm 123.8 and 227.2 \pm 97.5 pg/ml in 5 patients with AC, 256.5 \pm 46.2 and 298.9 \pm 172.5 in 4 patients with CAH and 166.6 \pm 62.9 and 146.8 \pm 45.1 in 13 control subjects.

However, a significant difference ($p < 0.05$) was found only for arterial PGE levels between AC patients and controls.

COMMENT

Our results first suggest that CAH lasts for a long period without evidence of water and salt retention, high levels of BUN and creatininemia: at this stage clinical signs or symptoms of cirrhosis with portal hypertension are not generally detectable.

However, by using more sensitive tests, such as renal clearances, acid loading tests, renal selective arteriography and scan, mild alterations can be found.

These findings become more and more frequent and evident in cases with AC. The incidence of such alterations is similar to that observed by other Authors in cases with cirrhosis, both for RTA (Golding *et al.*, 1973; Conte e Semplificini, 1975) and RPF and GFR (Shear *et al.*, 1965; Baldus *et al.*, 1964).

Angiographic findings have been found quite similar to those observed by Epstein *et al.* (1970).

It is important to stress, however, that these alterations occur in early stages of CAH, even if with lower incidence and evidence, whereas they have never been observed in cases with CPH.

The direct injection of ^{99m}Tc -microspheres of human albumin into the renal artery led us to confirm the opening of intrarenal shunts already seen (Gentilini *et al.*, 1976) in a good percentage of patients.

The phenomenon, (even if its estimation is rather approximate), appeared more evident in cirrhotic patients with early clinical and functional signs of portal hypertension.

These shunts may be renal AV shunts situated between cortex and medulla or abnormal dilatations of Ludwig-Isaacs' arteriolas.

High levels of PGE_1 and PGE_2 have been also detected, both in venous and more so arterial renal blood. This finding can explain the arteriolar tone reduction of the juxtamedullar nephrons (Campbell, 1974) or an intrarenal hemodynamic redistribution.

The increase of PG could be correlated to renal haemodynamics and functional alterations which occur also in early stages of CAH.

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The hepatorenal syndrome (HRS)

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Progressive oliguric renal failure commonly complicates the course of patients with advanced hepatic disease. While this condition has been designated by many names including "functional renal failure", and "the renal failure of cirrhosis", the more appealing albeit less specific term "hepatorenal syndrome" has been utilized commonly to describe this syndrome. For the purposes of this discussion, the hepatorenal syndrome may be defined as unexplained renal failure occurring in patients with liver disease in the absence of clinical, laboratory, or anatomic evidence of other known causes of renal failure.

CLINICAL FEATURES

A review of the clinical features of HRS reveals marked variability regarding both the clinical presentation and clinical course. The HRS occurs usually in cirrhotic patients who are alcoholic, although cirrhosis was associated with chronic alcoholism in only 50% of those who developed HRS in the Mayo Clinic series. Renal failure may develop with great rapidity, occasionally occurring in patients in whom normal GFR and concentrating ability has been previously documented within a few days of onset of HRS. While renal failure has been reported to follow events which reduce effective blood volume including abdominal paracentesis, vigorous diuretic therapy and gastrointestinal bleeding, it can occur with equal frequency in the absence of an apparent precipitating event. Virtually all HRS patients have ascites which is often tense. The degree of jaundice is extremely variable, and it is noteworthy that occasionally renal failure may develop at a time when the serum bilirubin concentration is decreasing. Most patients have a modest decrease in systemic blood pressure, but significant hypotension occurs usually as a terminal event. The serum creatinine characteristically increased before the BUN in the series reported by Shear and coworkers, whereas the opposite was true in the Mayo Clinic series. These data emphasize the great variability in the HRS, both within and between series of patients. The majority of patients die within three weeks of onset of azotemia, although rare patients have survived for several months with mild azotemia. HRS patients manifest a rather characteristic urine excre-

tory pattern, voiding urine which is practically sodium-free and retaining the capacity to concentrate urine to a modest degree.

While classic HRS is usually associated with advanced cirrhosis of the liver, it has also been reported in patients with other liver diseases including acute hepatitis, and hepatic malignancy.

Although the majority of reports suggest that the HRS occurs in patients who manifest evidence of severe hepatocellular disease, it is quite apparent that the HRS can occur with minimal jaundice and with little evidence of severe hepatic dysfunction.

ATN (acute vasomotor nephropathy)

Although much attention has been directed to the HRS, it should be borne in mind that cirrhotic patients are not less vulnerable than non-cirrhotic patients to the development of ATN. Indeed, a review of several published series discloses that among liver disease patients who developed renal failure, the etiology of the renal failure was more commonly ATN than the HRS.

Finally, the association between obstructive jaundice and ATN merits comment. Dawson noted that of patients undergoing operation for the relief of obstructive jaundice, the incidence of acute renal failure was many times greater than that encountered in a comparable group of non-jaundiced patients. Dawson also noted that the greater the degree of jaundice, the greater the risk of ATN. The demonstration that the risk of ATN is higher in the most deeply jaundiced patients prompted an investigation of the mechanism(s) in the Gunn rat (a species unable to conjugate bilirubin). These studies suggest that circulating conjugated bilirubin was responsible for the increased proclivity of jaundiced animals to develop renal failure. It should be noted, however, that there is a lack of unanimity of opinion regarding the uniqueness of the association of biliary tract disease and ATN.

DIFFERENTIAL DIAGNOSIS

The abrupt onset of oliguria in a cirrhotic patient does not necessarily imply the presence of HRS. Prerenal causes are important to differentiate. Volume contraction or cardiac pump failure may present as a "pseudohepatorenal" syndrome. Furthermore, as already mentioned, it is not uncommon for patients with alcoholic cirrhosis to develop classic acute renal failure (ATN). In such instances the differentiation from HRS can be made readily by recognition of the precipitating event and by characteristic laboratory findings. Although occasional patients with frank ATN of the non-oliguric form may have low urinary sodium concentration, numerous investigators have demonstrated that the urinary sodium concentration exceeds 30 mEq/L in patients with oliguric ATN.

In summary, the finding of a low urinary sodium concentration in the presence of oliguric acute renal failure precludes the diagnosis of ATN. Only when pre-renal failure and ATN are excluded, can one establish the diagnosis of HRS.

PATHOGENESIS

Several lines of evidence have lent strong support to the concept that the renal failure in HRS is functional in nature. Despite the severe derangement of renal function, pathologic abnormalities are minimal and inconsistent. Furthermore, tubular functional integrity is maintained during the renal failure as manifested by a relatively unimpaired sodium reabsorptive capacity and concentrating ability. Finally, more direct evidence is derived from the demonstration that kidneys transplanted from patients with HRS are capable of resuming normal function in the recipient.

Despite extensive study, the precise pathogenesis of the HRS remains obscure. Many studies utilizing diverse hemodynamic techniques have all documented a significant reduction in renal perfusion. Since a similar reduction of renal perfusion is compatible with urine volumes exceeding one liter in many patients with chronic renal failure it is unlikely that a reduction in mean blood flow per se is responsible for the encountered oliguria.

Our laboratory has applied the ^{133}Xe washout technique and selective renal arteriography to the study of the HRS and demonstrated a significant reduction in both calculated mean blood flow as well as preferential reduction in cortical perfusion. In addition, cirrhotic patients manifested marked vasomotor instability which was characterized not only by variability between serial xenon washout studies but also by instability within a single curve. This phenomenon has not been encountered in renal failure of other etiologies. In addition, Epstein and coworkers carried out simultaneous renal arteriography to delineate further the nature of the hemodynamic abnormalities. Selective renal arteriograms disclosed marked beading and tortuosity of the interlobar and proximal arcuate arteries, and an absence of both distinct cortical nephrograms and of vascular filling of the cortical vessels. Postmortem angiography carried out on the kidneys of five patients studied previously during life disclosed a striking normalization of the vascular abnormalities with reversal of all the vascular abnormalities in the kidneys: The peripheral vasculature filled completely and the previously irregular vessels became smooth and regular. These findings provide additional strong evidence for the functional basis of the renal failure, operating through active renal vasoconstriction.

Although renal hypoperfusion with preferential renal cortical ischemia has been shown to underly the renal failure of HRS, the factors responsible for sustaining the reduction in cortical perfusion and the suppression of filtration

in HRS have not been elucidated. Several major hypotheses have been implicated or suggested, including: *a*) the renin-angiotensin system; *b*) alterations in intrarenal blood flow distribution; *c*) an increase in sympathetic nervous system activity; *d*) alterations in the endogenous release of renal prostaglandins; *e*) changes in the kallikrein-kinin system and the role of vasoactive intestinal peptide. It is not the purpose of this review to examine all these factors exhaustively. Rather, I would like to address my attention to an assessment of the role of two of the hormonal effectors for which recent evidence has been marshalled. The role of the renin-angiotensin system will be considered in detail by another speaker.

Role of Renal Prostaglandins

The possibility that prostaglandins participate in mediating the renal failure of cirrhosis must be considered. Initially, this possibility was assessed by examining the renal hemodynamic response to the administration of exogenous prostaglandins. Unfortunately, the relevance of such studies in cirrhotic man is tenuous since the physiological significance of PGA in both rabbit and man has been challenged. Furthermore, the interpretation of studies in which the effects of prostaglandin administration on renal function are examined, is difficult. The demonstration the PGE is almost completely inactivated in each passage across the lung suggests that any action of this lipid on the kidney must be as a local tissue hormone. Thus, any evaluation of the physiological role of prostaglandins on renal function necessitates an experimental design in which the endogenous production of the lipids is altered. Indeed, recent investigations of the role of prostaglandins on renal function have focused on comparisons before and after the administration of inhibitors of prostaglandin synthetase.

Zipser *et al.* have documented markedly elevated prostaglandin E₂ levels in 11 cirrhotic patients with avid sodium retention. The administration of inhibitors of prostaglandin synthetase (both indomethacin and ibuprofen) resulted in a lowering of PRA and PA and a 57% decrement in creatinine clearance. It is apparent that additional studies are necessary to characterize further the role of prostaglandins as determinants of the renal failure of cirrhosis.

Kallikrein-Kinin System

Several lines of evidence suggest that bradykinin and other kinins synthesized in the kidney may participate in the modulation of intrarenal blood flow and renal function. Experimental studies have demonstrated that intravenous infusion of kinins into humans or into the renal artery of dogs produces an increase in renal blood flow, urine flow, and urinary sodium excretion. That the kalli-

krein-kinin may indeed contribute to the renal failure of cirrhosis is suggested by studies of Wong *et al.* These investigators measured plasma prekallikrein levels in 7 patients with HRS and reported undetectable levels in 6 of the patients and a markedly low level in the remaining patient. The authors suggested that the decrease in prekallikrein levels results in diminished kinin formation. Since bradykinin has been suggested to be a physiological renal vasodilator, it is possible that failure of bradykinin formation may contribute to the renal cortical vasoconstriction encountered in HRS.

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The renin-angiotensin system in cirrhosis

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Renin and aldosterone levels are frequently increased in advanced cirrhosis (Schroeder *et al.*, 1970, Epstein *et al.*, 1977), particularly in patients with ascites and avid sodium retention (Rosoff *et al.*, 1975), but the mechanism leading to this hyperreninemia and hyperaldosteronism remains controversial.

It has been suggested that a defective hepatic metabolism of renin and aldosterone (Coppage *et al.*, 1962, Heacox *et al.*, 1967), a reduced renal perfusion (Schroeder *et al.*, 1970), an alteration of blood volume distribution and hemodynamic disturbances (Epstein *et al.*, 1977), and portal hypertension (Anderson *et al.*, 1976) may all be involved in the activation of the renin-angiotensin system in decompensated cirrhosis. In the present report, these concepts, as well as the possible role of the renin-angiotensin-aldosterone system as a determinant of sodium retention and of renal failure in cirrhosis, are reviewed on the basis of several studies performed in our institution (Arroyo *et al.*, 1977, 1978, 1979; Bosch *et al.*, 1976, 1977, 1978).

Frequency of increased renin and aldosterone levels: Relationship with sodium excretion

The results of the measurement of plasma renin activity (PRA) and plasma aldosterone concentration (PA) in four groups of patients with cirrhosis are summarized in Figure 1. As it is evident, PRA and PA are frequently increased in cirrhosis, but only in those patients with a very low urinary sodium excretion (Groups 3 and 4), while they were normal or only slightly increased in patients with ascites and less avid sodium retention (Group 2), and normal or even low in patients without ascites (Group 1). Thus, there is an inverse relationship between renin and aldosterone levels and sodium excretion. In fact, when patients with ascites are considered all together, a highly significant inverse correlation is observed between plasma aldosterone and the urinary sodium excretion, both plotted logarithmically ($r = -0.87$, $p < 0.001$) (Arroyo *et al.*, 1979). These results support the traditional view that hyperaldosteronism is an important factor in the pathogenesis of sodium retention in cirrhosis (Sherlock, 1975). However, a normal PA was found in a significant number of patients with ascites.

tes, indicating that sodium retention may be present in the absence of hyperaldosteronism. These results are in keeping with recent reports showing that in patients with ascites sodium retention may persist in spite of lowering PA to normal levels (Rosoff *et al.*, 1975; Epstein *et al.*, 1977), and suggest that factors other than aldosterone are also involved in the reduced sodium excretion of cirrhosis.

Hepatic metabolism of renin and aldosterone

Since both renin and aldosterone are mainly, if not sole, metabolized by the liver (Coppage *et al.*, 1962; Heacox *et al.*, 1967), the possibility that in cirrhosis a defective hepatic metabolism could contribute to increased levels of these substances should be considered.

We studied the hepatic metabolism of renin and aldosterone in cirrhosis by determining their respective concentration in arterial and hepatic venous blood. Multiplying the arteriovenous concentration difference per the hepatic plasma flow, which was simultaneously determined, it was then possible to calculate the hepatic clearance of renin and aldosterone (Bosch *et al.*, 1977). We have found that the hepatic clearance of renin was directly related to the plasma renin concentration, the liver removing more renin as plasma renin increased. These results suggest that an impaired hepatic metabolism is not the major determinant of increased renin levels in cirrhosis. On the other hand, in a series of cirrhotics in whom we measured the rate of renin secretion by the kidneys, we found that renin levels were in a close direct relationship with the renin secretion rate ($r = 0.93$, $p < 0.001$) (Bosch *et al.*, 1977; Arroyo *et al.*, 1979). So the results of our studies indicate that increased plasma renin in cirrhosis is mainly due to an increased secretion and not to a defective hepatic inactivation.

In relation to aldosterone, we found that the hepatic extraction was reduced in patients with ascites. However, the hepatic clearance of aldosterone was directly related to plasma aldosterone, the amount of aldosterone removed by the liver per minute being higher as higher were the plasma levels. This is the contrary to be expected if an impaired hepatic inactivation was the main factor leading to hyperaldosteronism. So, although the hepatic metabolism of aldosterone is reduced, this by itself does not allow to explain the high PA of these patients. On the other hand, the parallelism between PA and PRA shown in Figure 1, in which it is evident that patients with a high PRA also have a high PA, whereas those with a normal PRA had a normal plasma aldosterone concentration, illustrates the close direct correlation observed between the activation of the renin-angiotensin system in cirrhosis and the development of hyperaldosteronism (Arroyo *et al.*, 1979; Epstein *et al.*, 1977). Since PRA in cirrhosis has been found to parallel the plasma levels of angiotensin II (Kondo *et al.*, 1974),

a potent stimulus of aldosterone secretion, these findings suggest that increased aldosterone levels in cirrhosis reflect an increased aldosterone secretion induced by the activated renin-angiotensin system. Rossof *et al.* (1975), studying the metabolic clearance rate of labelled aldosterone in decompensated cirrhosis, also found that increased secretion is far more important than impaired metabolism in determining high aldosterone levels in cirrhosis.

The renin-angiotensin system and the renal perfusion

It has been suggested that increased renin levels in cirrhosis could be promoted by a reduction of the renal blood flow (Schroeder *et al.*, 1970, Barnardo *et al.*, 1970). However, this has not been confirmed in our studies (Arroyo *et al.*, 1979). As it is shown in Figure 1, although patients with renal failure (group 4) had a high PRA, the same was observed in non-azotemic cirrhotics with avid sodium retention (group 3). In this later group, PRA was much higher than in patients with ascites and relatively high sodium excretion (group 2) despite a similar renal plasma flow (RPF) and glomerular filtration rate (GFR). In the overall patients, no correlation could be established between plasma renin and the RPF or the GFR. Also, the rate of renin secretion by the kidneys was unrelated to the renal plasma flow (Arroyo *et al.*, 1979), thus giving further support to the concept that the renal perfusion is not the major determinant of renin secretion in decompensated cirrhosis.

On the other hand, since angiotensin II, the active component of the renin-angiotensin system, is a potent renal vasoconstrictor (Hollenberg *et al.*, 1972), it has also been suggested that the activation of the renin-angiotensin system may be involved in the pathogenesis of the functional renal failure of cirrhosis (Baldus, 1970), in which an active vasoconstriction of the renal arteries plays a critical role (Epstein *et al.*, 1970). However, our studies suggest that functional renal failure in cirrhosis could not be satisfactorily explained only by the vasoconstrictor effect of endogenous angiotensin II. This suggestion is based on the observation that some patients with ascites have a very high PRA in spite of a normal RPF and GFR (Figure 1, Group 3), and also on that the infusion of saralasin, a specific competitive antagonist of angiotensin II, to three patients with functional renal failure, failed to increase the RPF and GFR, in spite of achieving an effective blockade of the effects of angiotensin II (Bosch *et al.*, 1978). On the other hand, recent studies have shown that a deficient synthesis and release of renal vasodilators, such as renal prostaglandins (Boyer and Reynolds, 1976) and kallikrein (Wong *et al.*, 1977) may be important factors in decreased renal perfusion in cirrhosis. According to these observations it may be suggested that functional renal failure is the result of an imbalance between vasoconstrictor stimulus and renal vasodilator substances. When the later are

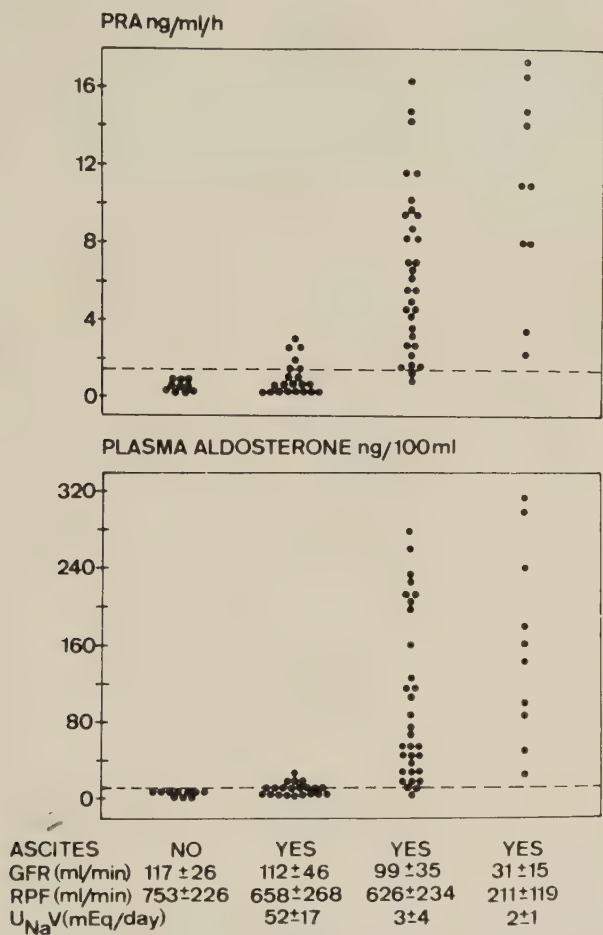


FIG. 1. Plasma renin activity and plasma aldosterone concentration in four groups of cirrhotics. The horizontal lines indicate the upper normal limit in our laboratory. Group 1 includes cirrhotics without ascites, group 2 includes non-azotemic cirrhotics with ascites and relatively high urinary sodium excretion (U_{Na}V), group 3 non-azotemic cirrhotics with ascites and very low U_{Na}V, and group 4 patients with functional renal failure. (GFR = glomerular filtration rate, RPF = renal plasma flow) (Mean ± SD).

adequate, an excess of endogenous vasoconstrictors such as angiotensin II, does not result in renal failure. This however will occur if there is a defective synthesis of renal vasodilator substances. This hypothesis is supported by the observation of some patients with ascites in whom renal failure has followed the administration of non-steroidal antiinflammatory drugs, potent inhibitors of prostaglandin synthesis (Arroyo *et al.*, 1978).

Relationship between the renin-angiotensin system and general hemodynamics

For many years it has been known that arterial hypertension occurs in a significantly lower incidence in cirrhosis than in the general population (Schwartz, 1967). In fact the arterial pressure of patients with cirrhosis and ascites is significantly lower than that of age and sex matched controls (Fig. 2). Since hypo-

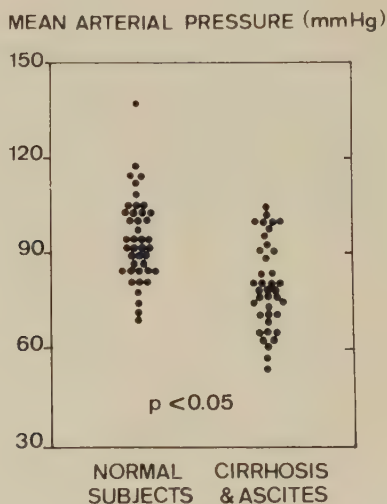


FIG. 2. Mean arterial pressure in cirrhotics with ascites and in a control group of healthy subjects.

tension is a potent stimulus of renin release, the suggestion can be raised that the activation of the renin-angiotensin system in cirrhosis could be a homeostatic response to reverse a state of systemic hypotension. We tested this hypothesis by infusing the synthetic competitive antagonist of angiotensin II, saralasin, to 14 patients with ascites, in order to block the effects of endogenous angiotensin II (Arroyo *et al.*, 1977). As in the study of Schroeder *et al.* (1976), saralasin infusion induced a decrease of the arterial pressure, which ranged between mild to marked degrees of hypotension. These results indicate that endogenous angiotensin II is active in support of blood pressure in cirrhosis. The percent decrease of the arterial pressure induced by saralasin infusion was directly related to the baseline PRA ($r = 0.70$, $p < 0.01$). These findings support the hypothesis that a preexisting arterial hypotension could be the stimulus promoting renin secretion in cirrhosis with ascites, in an attempt to restore the arterial pressure to normal levels.

The mechanism by which hypotension would develop in advanced cirrhosis is not clear, but it may be related to changes in the hepatic hemodynamics, since we have found a close direct correlation between the wedged hepatic vein pressure—which reflects increased resistance to hepatic blood flow at sinusoidal or postsinusoidal sites—and the plasma renin levels (Bosch *et al.*, 1976). Several mechanisms may be suggested to explain the relationship between increased intrahepatic pressure and renin secretion, including humoral (Orloff *et al.*, 1964), neurogenic (Anderson *et al.*, 1976) and hemodynamic changes. We have payed particular attention to this later possibility since in cirrhosis there is usually an increased cardiac output and plasma volume (Tristani and Cohn, 1967, Lieberman and Reynolds, 1967), and disturbances of blood volume distribution

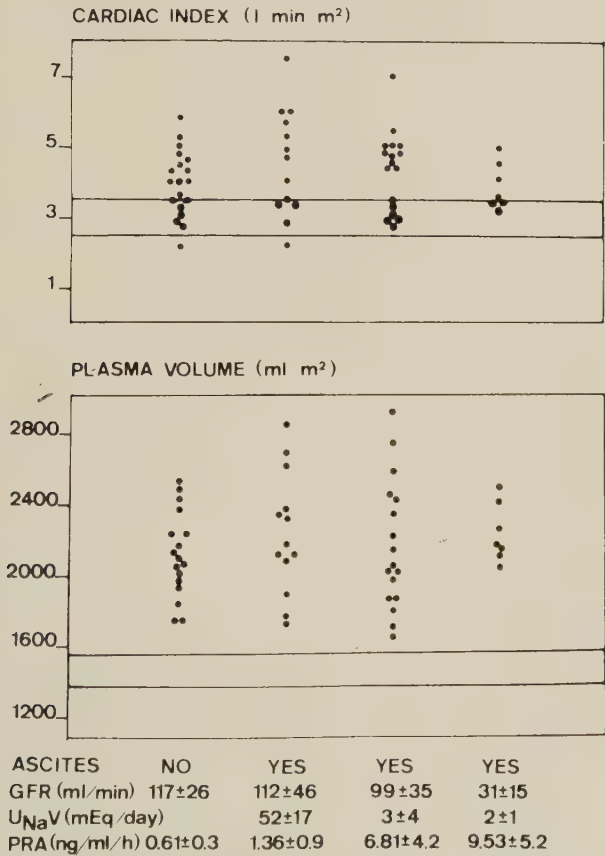


FIG. 3. Cardiac output and plasma volume in four groups of patients with cirrhosis of the liver. The shaded area represents the normal range in our laboratory (same groups than in figure 1) (PRA = plasma renin activity) (Mean ± SD).

have been related to the activation of the renin-angiotensin system in these patients (Epstein *et al.*, 1977). The results of the measurement of the cardiac index and plasma volume in the four previous groups of cirrhotics are summarized in Figure 3. As it is shown in the figure, the cardiac output and plasma volume were in average increased to a similar degree in the four groups, independently of the presence of ascites, of renal function and of renin levels. The apparent lack of correlation between these parameters and the activation of the

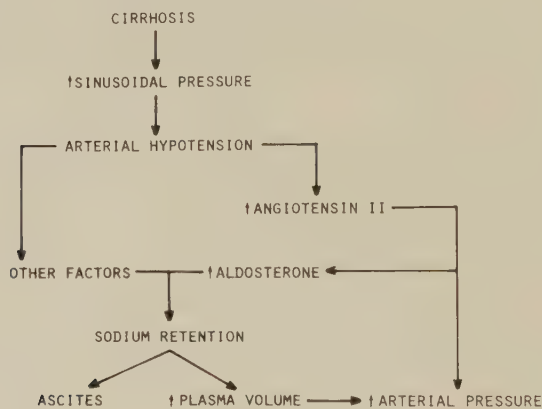


FIG. 4. Scheme of the pathogenesis of ascites (see text).

renin-angiotensin system is probably due to compensatory hemodynamic adjustments, directed to reverse a state of systemic hypotension. We suggest that in cirrhosis there is a peculiar hemodynamic disturbance, probably related to the increased intrahepatic vascular resistance, characterized by arteriolar vasodilatation and concomitant arterial hypotension. In earlier stages of cirrhosis, this hemodynamic disturbance could be counterbalanced effectively by an expansion of the plasma volume and by a concomitant increase of the cardiac output. However, in more advanced stages, as the wedged hepatic vein pressure increases, ascites formation precludes an effective expansion of the plasma volume, and hypotension could only be reversed by an activation of the renin-angiotensin system. At this time, endogenous angiotensin II would be the main factor supporting blood pressure. Increased aldosterone levels would be a secondary event, contributing to both the expansion of the plasma volume and to the accumulation of ascites. This hypothesis is depicted in Figure 4.

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The role of endotoxins in renal failure in chronic liver disease

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Since Levin *et al.* (1970) evaluated the Limulus Gelation Test for detection of endotoxins in biological fluids evidence accrued by a number of investigations (Caridis *et al.*, 1972; Clemente *et al.*, 1977; Grün *et al.*, 1977; Prytz *et al.*, 1976; Tarao *et al.*, 1976; Wardle 1974; Wilkinson *et al.*, 1974, 1976) that endotoxemia is a frequent complication of both, acute and chronic liver disease. By their multiple biological activities certain extrahepatic events may be linked to circulating endotoxins (for rev. Liehr and Grün 1977; Nolan 1975) including renal failure. The concept has the following basis. Administration of bacterial endotoxins to laboratory animals leads to vasoconstriction of the kidney with subsequent decrease of renal function (Cavanagh *et al.*, 1970; Gillenwater *et al.*, 1963). Fritz (1973) described a cytotoxic effect of endotoxins on renal tubular cells. Mitochondrial metabolic alterations of tubular cells were found as subsequent to endotoxin administration (Mela, 1975). Bonisou *et al.* (1968) mentioned that endotoxins by triggering disseminated intravascular coagulation may cause both full cortical necrosis or merely tubular necrosis via capillary thrombosis. In man, the administration of typhoid endotoxin results in intrarenal vasoconstriction and decrease of urine flow (Lathem, 1956). Since these alterations resemble changes of renal disorders in acute and chronic liver disease of man (for rev. see Wilkinson, 1977) the hypothesis was put forward that endotoxemia may be contributive to or even causative of renal damage in liver disease (Wardle, 1974). Support can be given by both experimental and clinical data.

EXPERIMENTALLY

Grün *et al.* (1976) investigated the effect of a nonlethal dose of *E. coli* endotoxin administered to rats. Although total renal blood flow did not change significantly by means of cardiac output fraction a profound alteration of intrarenal blood flow distribution, i.e. a cortico medullary shift was observed. The increase of medullary perfusion on account of renal cortical blood flow was found more marked, if a threefold higher dose of endotoxin was administered (Grün, 1978). Short after endotoxin injection urine osmolality decreased to

values being the fifth of normal osmolality. Decrease of urine sodium concentration outlasted these early endotoxin effects, and was thought due to tubular necrosis which latter were found dose dependent in number by lightmicroscopy. Thus, endotoxins may cause intrarenal hemodynamic disorders, subsequent functional alterations, and even anatomical damage of tubular cells. In models of acute and chronic liver disease, such as in galactosamine hepatitis in rats and in the portacaval shunt rat, endogenous endotoxemia was found in 78% and 88%, respectively (Grün and Liehr, 1977). The evaluations of renal function in these models resulted in the finding that spontaneously a cortico medullary shift is present in these rats. Since no shock equivalents are present in these animals and the renal alterations were found comparable to events observed after endotoxin administration to otherwise healthy animals, systemic endotoxemia was incriminated as causative (Grün *et al.*, 1976; Grün, 1978). Exogenous endotoxemia imposed to endogenous endotoxemia in portacaval shunt rats resulted in a much more marked renal damage comprising both intrarenal vascular thrombosis and full cortical necrosis (Grün *et al.*, 1976). Comparable results were obtained by Wolter *et al.* (1977) in minipigs. The explanation was given that an active portacaval collateral circulation enhances endotoxin toxicity because of hemodynamically induced failure of Kupffer cells (Liehr *et al.*, 1975).

CLINICALLY

Renal damage in liver disease is matter of debate since Flint (1863) reported oliguria in liver cirrhosis beside normal renal histology. Vesin (1962) introduced the term of functional renal failure determined as a state of low sodium excretion, oliguria and increase of blood urea nitrogen. Wilkinson *et al.* (1974) demonstrated in patients with fulminant hepatic failure that such alterations occur in close correlation with systemic endotoxemia which latter was detected by means of the Limulus Gelation Test (Levin *et al.*, 1970). Such designed correlation studies were also done in patients with liver cirrhosis (Clemente *et al.*, 1977; Liehr *et al.*, 1976; Wardle, 1974; Wilkinson *et al.*, 1976), and comparable results were obtained. Wilkinson (1977) suggested that in both diseases endotoxin induced vasoconstriction of the kidney may cause merely functional renal failure. If endotoxemia is more marked, and intravascular fibrin depositions because of intravascular disseminated coagulation are present, as proposed by Wardle (1976), tubular necrosis may develop. Beside these correlation studies a more crucial study will be to treat endotoxemia in cases with accompanying renal failure. An agent which seems suitable in this context is polymyxin B (Neter *et al.*, 1958) destroying the endotoxin molecule on a molecular basis (Lopez and Inniss, 1969). A such designed study was done (Sautter *et al.*, 1977) using a dose of polymyxin B (Pfizer, Karlsruhe, W. (Germany) of 1.1 mg/kg b.w.

over a period of three days. A rapid increase of both urine volume and sodium excretion was found. Comparable results were mentioned by Wilkinson *et al.* (1976). Thus, endotoxins are potent agents to induce renal failure, which may be functional on the one side of the spectrum and due to anatomical disorders at the end of spectrum (Wilkinson, 1977). Furthermore, no real difference in the type of endotoxin induced renal failure seems to be present in both, acute and chronic liver disease, once endotoxemia has developed. The grade of renal failure obviously is determined by the grade of endotoxemia. Since related therapeutical action will be effective only during the functional state, early recognition of both endotoxemia and renal failure is necessary. The latter may be done by consecutive measurement of urine sodium excretion.

CONCLUSION

The introduction of endotoxemia as causative of renal disorders in acute and chronic liver disease is a new concept. Since by both, experimental and clinical data, some reasonable support is provided, a more rational approach is given to understand better renal disorders in liver disease. At least, the concept seems a valid basis for future investigations in order to have an effective clinical action.

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Impairment of the kidney in experimental liver damage

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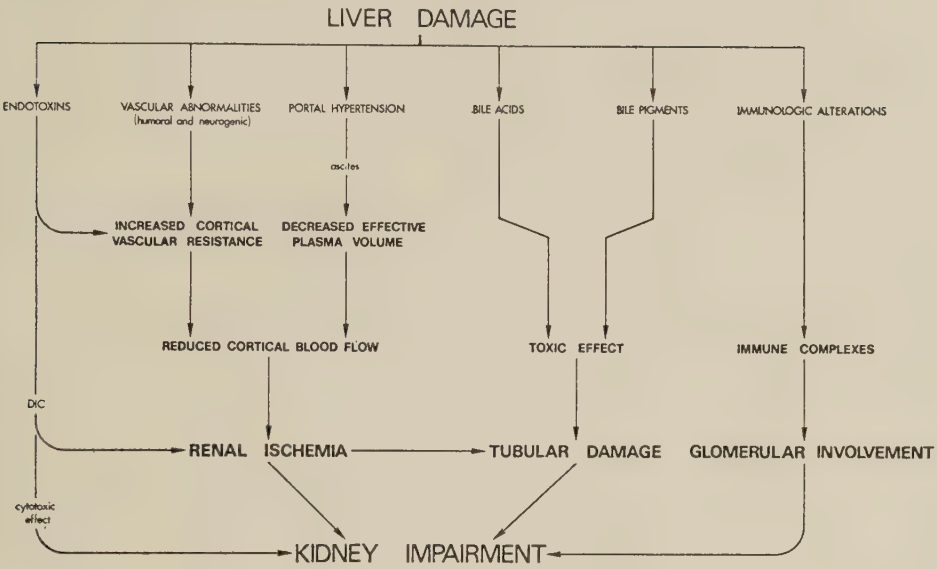
Clinical and experimental studies have recently provided further evidence for the existence of definite relationships between liver and kidney pathology. The pathophysiologic characters of the damage affecting the kidney in the course of hepatobiliary diseases have to be considered on the basis of toxic, hemodynamic, metabolic and immunologic factors which, singularly or variously associated, may act (Table 1).

The basic role of bacterial endotoxins in the pathogenesis of renal impairment has been proved by a large number of experimental studies (14). The presence of systemic endotoxemia has been detected in several hepatic diseases, mainly as a consequence of either an increased intestinal absorption and/or a decreased hepatic clearance by Kuppfer cells (3, 17). Endotoxins exhibit multiple remarkable biological activities which account for their ability to damage the kidney by producing both direct cytotoxic effects and coagulatory and vascular alterations (16), as elucidated by Prof. Liehr in his lecture. Moreover, according to our investigations elsewhere presented in this paper, endotoxins can stimulate specific immune response, leading to an increase in circulating immune complexes (ICs) with the possible consequence of their glomerular deposition.

Abnormal humoral and neurogenic stimuli, as detected in several liver diseases, may produce severe disturbances in systemic or local circulation; therefore the possible occurrence of an intrarenal hemodynamic derangement must be emphasized. The following topics are worth mentioning:

- increased activity of the sympathetic nervous system and adrenergic activation;
 - accumulation of false neurotransmitters at sympathetic nerve endings (10);
 - alterations in renin-angiotensin system (1, 15);
 - increase in serum prolactin levels (12);
 - abnormalities in kallikrein-kinin and prostaglandin metabolism (11, 18).
- The end result might be an increase in cortical vascular resistances as a con-

TABLE 1



sequence of active renal vasoconstriction (9, 13). In addition, portal hypertension, when present, may lead to splanchnic pooling of blood, thus reducing the effective plasma volume.

A real interest in the possible role of bile acids (BA), as a cause of renal damage, stems from several studies concerning BA toxicity, either on cell membranes or on some metabolic pathways. It is well known that BA [reviewed by Emanuelli G. *et al.* (7)]:

- exhibit detergent-like properties and disrupt cell membranes;
- produce hemolysis *in vitro* (Berliner and Schoenheimer, 1938);
- inhibit ATP activity, oxygen uptake and protein synthesis in rat jejunum *in vitro* (Parkinson and Olson, 1964);
- disrupt lysosomes and cause acid hydrolases to be released *in vitro* (Weissman, 1965);
- interact with microsomal constituents, namely cytochrome P-450 (Hutterer *et al.*, 1970);
- inhibit liver-like anion transport system in rabbit kidney, uvea and cho-roid plexus (Barany, 1974);
- increase permeability of aortic endothelium (Wardle, 1975).

Finally BA render the kidney more susceptible to ischemic injury and are

able to produce ultrastructural changes of proximal convoluted tubules in isolated perfused rat kidney.

Unconjugated bilirubin (UB) exhibits various toxic effects on the cell, mainly by reducing respiratory activity and mitochondrial function. In synthesis, UB [reviewed by Emanuelli *et al.* (7)]:

- uncouples oxidative phosphorylation in mitochondria *in vitro* (Zetterstrom and Ernster, 1956);

- decreases uptake of aminoacids in tissue culture systems (Cowger *et al.*, 1965);

- interferes with electron transport system and depress cell respiration (Cowger *et al.*, 1965);

- inhibits membrane-bound respiratory enzymes in mitochondria (Cowger *et al.*, 1965);

- causes alterations in mitochondrial membrane permeability and induces mitochondrial irreversible swelling *in vitro* (Mustafa *et al.*, 1969);

- inhibits many NAD-dependent dehydrogenases such as glutamate (Yamaguchi, 1970), malate (Noir *et al.*, 1972) and isocitrate (Ogasawara *et al.*, 1973).

In vivo nephrotoxic effects of unconjugated hyperbilirubinemia have been studied in homozygous Gunn rat and mutant Southdown sheep [reviewed by Emanuelli *et al.* (7)]; the nephropathy of these natural models is characterized by functional disturbances, such as failure of renal concentrating ability, reduced glomerular filtration rate and renal plasma flow which are definitely related to morphologic lesions. The kidney damage consists in progressive and chronic medullary lesions: the diffuse bilirubin deposition in the papilla and medulla is followed by cytoplasmic degenerative changes and loss of cells by necrosis, first of interstitial cells, loops of Henle and vasa recta, and later of collecting ducts.

The effects of conjugated bilirubin (CB) on parenchymas have been rarely investigated, since it is difficult to obtain the pigment in pure form for experimental purposes. Nevertheless, CB seems to render the kidney more susceptible to ischemic noxa. In Wistar rat, subjected to common bile duct ligation, ischemic insult indeed produces severe lesions of convoluted tubules (4), while in Gunn rat, under the same experimental conditions, only mild tubular lesions can be detected (2).

Such results seem to disagree to the demonstrated toxicity of UB on cell metabolic systems, as it has been previously described. However, the most attractive suggestion on this point stands upon the different intrarenal fate of the two forms of pigment: UB is so tightly bound to albumin that it is undialyzable, while CB, hydrosoluble and less firmly bound, is filtered through the

glomerulus. Its presence in renal tubular lumen may obviously account for the entry into the tubular cell.

Impressive evidence for the ability of CB in exerting renal damage has been also provided by our investigations in experimental obstructive jaundice, pointed on lysosomal activity and respiratory function of tubular cell (8). In such condition, the massive entry of CB into the tubular cell is able to determine lysosomal damage and, therefore, acid hydrolase release. The cause of lysosomal injury is not clear, but a relation is evident between the increase of serum and tissue bilirubin and the release of lysosomal enzymes. Anyway, lysosomal lesion may lead to cytolysis processes and to indirect mitochondrial damage. Furthermore, it may be suggested that CB directly depresses mitochondrial respiratory activity as well as UB does; moreover it seems likely to assume a bilirubin intracellular deconjugation, operated by a β -glucuronidase release from lysosomes.

Finally, the results of recent experimental and clinical investigations have accrued dealing with the involvement of immune mechanisms in the pathogenesis of renal damage occurring in liver diseases. Our previous studies, using immunofluorescence (IF) procedure, revealed the presence of glomerular Ig and complement deposits in a series of patients with obstructive jaundice (6). The immunologic glomerular involvement has been experimentally observed in rat subjected to common bile duct ligation. Early (3,5 days) IgG deposits have been detected in mesangium and subendothelial space by IF technique; later a progressive increase of IgG deposits and the appearance of IgA, IgM, C₃ and fibrinogen have been observed (Table 2).

In the same experimental condition, we have also evaluated different humoral immunologic characters such as systemic endotoxemia, anti-endotoxin antibodies (Ab), circulating ICs, polymorphonuclear leukocyte (PMN) phagocytosis and serum total complement levels. The presence of systemic endotoxemia and anti-endotoxin Ab has been observed; circulating ICs, yet present to some extent in many untreated control animals (but always in absence of any Ig glomerular deposit), showed a significant increase together with a gradual and progressive blockade of PMN phagocytosis (Table 2).

Similar results have been obtained in experimental intrahepatic cholestasis, induced by α -naphthyl isothiocyanate (ANIT). Humoral data, including systemic endotoxemia, anti-endotoxin Ab, circulating ICs and PMN phagocytosis, are superimposable in regard of the extrahepatic cholestatic condition (Table 3).

Finally a similar glomerular IF pattern has been detected in acute toxic hepatitis, experimentally induced by D-galactosamine administration, and related to the presence of systemic endotoxemia, early demonstrated in this condition.

The significance of glomerular immunologic involvement, as we observed in

TABLE 2

Glomerular immunofluorescence findings and humoral immunologic parameters in rat obstructive jaundice induced by common bile duct ligation

Days	Animals No.	Glomerular IF			Systemic Endotoxemia *	Anti- Endotoxin Ab	PMN ICs **	PMN Pha- gocytosis % ***	Complement CH ₁₀₀ U./ml
		IgG	IgA	IgM					
Control	9	0	0	0	0	0	present	95	41.3 ± 8
3	5	4/5	0	0	5/5	1/5	increased	40	23.0 ± 5
5	5	4/5	0	0	3/5	2/5	increased	40	32.9 ± 7
7	5	5/5	0	1/5	3/5	4/5	increased	38	28.5 ± 4
10	5	5/5	2/5	3/5	3/5	5/5	increased	20	44.3 ± 8
15	5	5/5	1/5	2/5	2/5	5/5	increased	15	31.2 ± 6

* Systemic endotoxemia has been detected by "Limulus gelation test" (Levin, J. *et al.*, 1970; *J. Lab. Clin. Med.* 75, 903).

** Circulating immune complexes have been evaluated as ICs bound to polymorphonuclear membrane receptors (Camussi G. *et al.*, 1978; *Int. Arch. All. appl. Immunol.*, in press).

*** Polymorphonuclear phagocytosis has been evaluated as ability of PMN to phagocyte complement activated particles of zymosan (Camussi, G. *et al.*, 1978; *Int. Arch. All. appl. Immunol.*, in press).

TABLE 3

Glomerular immunofluorescence findings and humoral immunologic parameters in rat intrahepatic cholestasis induced by alpha-naphthyl isothiocyanate (ANIT) administration (Desmet, V. *et al.*, 1968: *Am. J. Path.* 52, 401)

Days	Animals No.	Glomerular IF				Systemic Endotoxemia *	Anti- Endotoxin Ab	PMN ICs **	PMN Pha- gocytosis % ***	Complement CH ₁₀₀ U./ml
		IgG	IgA	IgM	C ₃					
Control	9	0	0	0	0	0	0	present	95	41.3 ± 8
	5	1/5	0	0	0	5/5	1/5	increased	50	38.0 ± 17
	5	1/5	0	0	0	4/5	1/5	increased	16	43.2 ± 12
	10	4/5	0	0	0	4/5	3/5	increased	36	61.9 ± 29

* Systemic endotoxemia has been detected by "Limulus gelation test" (Levin, J. *et al.*, 1970: *J. Lab. Clin. Med.* 75, 903).
** Circulating immune complexes have been evaluated as ICs bound to polymorphonuclear membrane receptors (Camussi, G. *et al.*, 1978: *Int. Arch. All. appl. Immunol.*, in press).
*** Polymorphonuclear phagocytosis has been evaluated as ability of PMN to phagocyte complement activated particles of zymosan (Camussi, G. *et al.*, 1978: *Int. Arch. All. appl. Immunol.*, in press).

the course of experimental liver damage, is still matter of speculation. Firstly the behaviour of Ig deposition supports a critical suggestion on this point. The early detected Ig deposits (observed at 3,5 days) may be related to abnormal glomerular trapping of pre-existing ICs, since RES clearance ability is decreased in obstructive jaundice and other liver diseases (5). By this regard, the significant inhibition of PMN phagocytosis seems well in agreement with this statement. The progressive increase in glomerular Ig deposits, as observed in later stage of obstructive jaundice, also suggests that an immune response against exogenous antigens might be stimulated in the presence of anatomic and functional liver damage. The occurrence of systemic endotoxemia and the presence of anti-endotoxin Ab seem to suggest that bacterial endotoxins actually play a role in producing such response.

In conclusion, our investigations demonstrate by IF microscopy the kidney immunologic involvement in obstructive jaundice and other related experimental conditions primarily affecting the liver. The pathogenetic significance of immunoglobulins in glomeruli, as we observed, has to be demonstrated. The evidence for the operation of an immune complex type of injury is lacking; abnormalities of the general and basic immunologic mechanisms, such as the source and clearance of ICs and the systemic immune response, are likely responsible and primarily involved.

Anyway the pathophysiologic role of the liver must be stressed since functional exclusion of the organ, as we obtained in experimental portal hypertension and portocaval anastomosis, produces similar glomerular features and humoral immunologic abnormalities including subendothelial-mesangial Ig deposits and the presence of systemic endotoxemia and anti-endotoxin Ab.

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SYMPOSIA
AIR POLLUTION

Introduction

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This symposium is devoted to one of the most interesting problems of Internal Medicine, namely to "Air Pollution and Respiratory Diseases".

As everyone knows, in addition to air pollutants of natural origin (pollens, different products of the vegetable kingdom, and others), there also exist other very important pollutants of artificial source, connected with the activities of man and directly linked to the industrial development.

These air pollutants are various in type (vapours, fumes, dust, smog, micro-molecular substances, and others); however, the levels of atmospheric pollution, especially in some urban areas and in some industrial environments, are very high and can cause several diseases of the respiratory tract, rhinitis, (pharyngitis, tracheitis), bronchitis, alveolitis, of different severity and length.

Air pollutants, according to their noxious properties, can be divided into: a) irritating agents; b) sensitizing agents; c) fibrosing agents; d) agents provoking a granulomatous response.

I would like to underline, in these introductory remarks, just a few aspects of the respiratory diseases produced by atmospheric pollution, such as obstructive lung disease, extrinsic allergic alveolitis and fibrosing alveolitis. All these subjects will be shortly discussed by the Speakers of this Symposium.

As a preliminary statement, I think that special stress should be laid on the great difficulties we meet in the carrying-out of *epidemiological studies* on respiratory diseases due to air pollutants. It is in fact quite difficult, if not nearly impossible to extrapolate, from among a large number of risk factors, the effect of a long term exposure to an air pollutant. A paper of Prof. Gandevia of Australia was scheduled to be presented in this Symposium. Unfortunately, he was unable to attend. However, the guidelines of his paper can be found in his abstract and I quite agree with him on the risk of a misuse of epidemiological data.

It may be observed, on the other hand, that even low levels of air pollutions can prove noxious to subjects with an aspecific bronchial hyperreactivity, either congenital or acquired, as in some physiopathological states, especially characterized by a partial blockade of β_2 -adrenergic receptors or by excessive cholinergic responses namely, by a deranged autonomic nervous control.

The *obstructive lung disease*, transient or chronic, more or less reversible, is one of the most frequent disorders produced by air pollution; in this regard, I would like to emphasize the ethiological importance of many irritating and/or sensitizing micromolecular substances.

The prolonged exposition to such substances can especially produce in many cases an impairment of the small airways, as Prof. Yokoyama will report today. On the other hand, I would like to point out that also some studies we made, conclusively revealed that a precocious impairment of small airways (of a diameter lower than 2 millimeters), also without any symptoms of respiratory distress, were clearly apparent in about 50% of non-smoking subjects suffering from disorders of the upper respiratory tract, vasomotor allergic rhinitis in particular.

The mechanism of the obstructive lung disease produced by air pollutants can be immunological or non immunological in the latter case, it is quite possible that the disorder of bronchial vagal irritant receptors may play a role of primary importance, possibly with an aspecific release of chemical mediators which was recently attributed to the activation of the complement's alternative pathway.

Many important advances were made in recent years on the *extrinsic allergic alveolitis*, and this was particularly due to the very interesting studies of my friend, Prof. Jack Pepys of London.

As you may know, since the first description of the farmer's lung, in the year 1932, many other types of allergic alveolitis were described and many aetiological agents were identified: thermoresistant spores of mycetes, antigens from mites, heterologous proteins of avian and other origins and, quite recently, free-living amoebae. Type III immune reactions, and, to a lesser extent, type IV reactions, are especially involved in these disorders [however, in some types of alveolitis the intervention of type I and type II immune reactions can be proved].

Fibrosing alveolitis, on the contrary, is produced by inhaled inorganic particles (of silic, asbestos, and others), which can give a diffuse pulmonary fibrosis, either directly or by provoking a granulomatous response; as Prof. Blasi and Prof. Olivieri of Naples will point out, an impaired relationship between alveolar macrophages and fibroblasts, can be at the basis of the increased synthesis of collagen fibres.

Another interesting problem concerns the *prevention of air pollution*; some aspects of this topic will be illustrated by Prof. Reinberg of Paris also on behalf of Dr. Gervais especially in regard of the prevention of atmospheric pollution in highly populated and heavily air-polluted cities.

From a general point of view, such the well-known preventive individual measures can be considered, as the removal of the patient from exposure to

irritating and/or sensitizing substances. This can be done by changing his working activity or his work environment, or, in some particular cases, by resorting to the use of masks. More difficult are the preventive measures of a general character, which represent quite a serious problem for the Public Health Authorities.

But the time allotted to this Symposium is not very long. So I think we would better start our works right now: other interesting problems may arise from the discussion that will follow the lectures.

Epidemiology of respiratory diseases due to air pollution

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In transient extreme conditions of urban atmospheric pollution, an excess of mortality from chronic respiratory diseases has been adequately demonstrated (cancer is excluded from this discussion). To extrapolate from this to suggest that chronic morbidity, and especially disability, from these disorders is attributable to the levels of atmospheric pollution prevailing in cities today is unjustified. Even if excess bronchial secretion results from long continued exposure to urban air, this does not imply that disabling airways obstruction supervenes. This generalisation is also true of smoking, which involves greater levels of personal air pollution. Differences in urban/rural mortality and morbidity ratios involve so many factors (social, demographic and medical) that it is impossible to isolate an effect of long-term exposure to urban air. Studies of non-smoking occupational groups (with some reservations) do not reveal excess significant morbidity from chronic non-specific obstructive syndromes; despite qualitative differences, industrial pollutant levels are generally higher than in urban air.

Acute experiments and animal experiments are not relevant to chronic human disease. To examine the relationship of chronic obstructive syndromes to contemporary atmospheric pollution would require long-term prospective epidemiological studies, commencing in childhood and employing more sophisticated methodology than is conventionally used. The cost of an appropriate investigation is prohibitive in itself, but the inevitable lapse rate and the practical difficulties of defining an appropriate control population also render the concept scientifically unrewarding. Occupational populations are relatively small, well-defined and stable, personal pollution and smoking levels can be controlled and sophisticated respiratory tests can be applied. If industrial pollution in the working environment produced no excess of mortality or disabling respiratory disease, the socio-economic consequences of control of environmental pollution might be minimised.

This paper is directed against the misuse of epidemiological resources and of scientific data, and not against the control of environmental pollution for aesthetic reasons or to control acute reactions.

Air pollution and obstructive lung diseases

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INTRODUCTION

In the last fifteen years status of air pollution in Japan has remarkably changed both in quality and in quantity. During this period feature of the airborne pulmonary diseases made considerable change which presumably be related to the changing feature of air pollution. Focus of the clinical attention on the airborne pulmonary diseases was also changed from the central airway obstruction, which was characterized by chronic obstructive lung diseases, to the distal airway obstruction or even to the damage of alveoli.

1. *Status of air pollution in Japan, past and present*

Annual mean SO₂ concentration in the industrialized area in Japan was 0.059 PPM in 1967, which was gradually decreased to 0.020 PPM in 1976. Environmental NO₂ concentration, which was represented by the annual mean of 0.026 PPM for 1968, demonstrated gradual increase in the following few years and was suppressed to maintain its level of approximately 0.033 PPM since 1973. In Tokyo Metropolitan area environmental CO concentration revealed tendency to increase until the year of 1965 and then started to decrease since 1970.

2. *“Bronchiolitis”*

The “bronchilitis” implies to the damage limited in the terminal airways and/or alveoli. Patients with subjective complaints of persistent cough and shortness of breath were tentatively described as “bronchitics”. Subjects with pulmonary diseases other than bronchitis or “bronchiolitis” were excluded from this category. From this group of the patients subjects with “bronchiolitis” were chosen on the clinically established criteria. The patients with “bronchiolitis” may produce small amount of mucous or does not produce it. The patients often complain tightness of chest or chest pain and cyanosis. On their chest X-ray disseminated nodular densities are usually demonstrated. On pulmonary function tests the patients may not demonstrate predominant ventilatory impairment detected either by %VC or by FEV1 % although they usually reveal

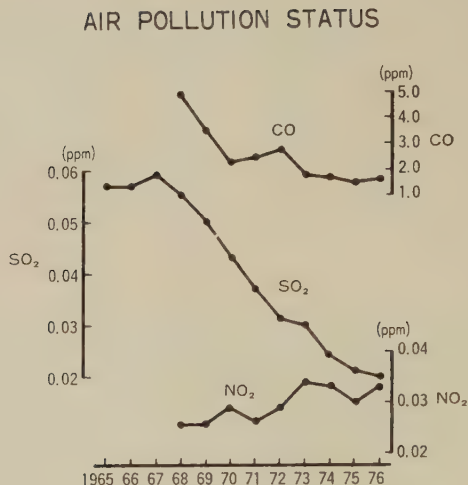


FIG. 1. Air pollution status in Japan. This figure was plotted based on the data released by the Environment Protection Agency for the Environment White Paper 1978.

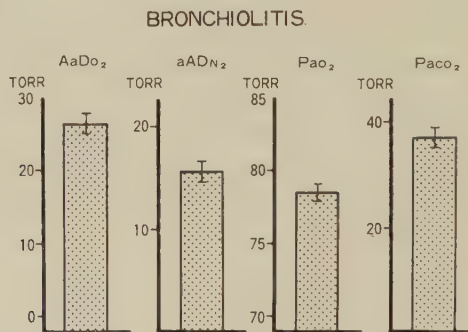


FIG. 2. Arterial blood gas data obtained on patients with "bronchiolitis".

arterial hypoxemia associated with abnormally increased AaDO₂ and aADN₂, and respiratory alkalosis. Arterial blood gas data obtained on 92 cases with "bronchiolitis" were 78.41 ± 0.78 torr (mean \pm 1 SE) for PO₂, 39.13 ± 0.34 torr for PCO₂, 25.69 ± 0.78 torr for AaDO₂ and 15.16 ± 0.51 torr for aADN₂. The mechanism to cause arterial hypoxemia was explained mainly by uneven distribution of ventilation-perfusion ratio in the lungs.

20.4% of cases with "bronchiolitis" studied demonstrated arterial O₂ tension below 70 torr although only 9.7% of cases with bronchitis revealed arterial O₂

tension below 70 torr. Prevalence of “bronchiolitis” in our clinic of Keio University Hospital, which is located in the Downtown Tokyo, in the last 10 years was studied. The prevalence for “bronchiolitis” among the whole pulmonary patients was 11.8%, which was twice of that for chronic pulmonary emphysema and was higher than that for bronchial asthma. The patient does not respond to bronchodilator although he responds well to steroid therapy.

On patients of “bronchiolitis” with AaDO₂ larger than 20 torr the prevalence of audible small bubbling rale or crepitation was 65.8% while on patients with AaDO₂ less than 10 torr it was only 21.1%. Prevalence of disseminated nodular densities on chest X-ray for the patients with “bronchiolitis” was also studied. For the patients with AaDO₂ larger than 20 torr prevalence was 68.5% while for those with AaDO₂ less than 10 torr it was 28.9%.

3. Annual decline of prevalence, obstructive bronchitis and “bronchiolitis”

From 1967 to 1976 we have done both spirometry and arterial blood gas study on 786 bronchitics.

Ratio of numbers of cases accompanied by abnormal FEV₁% against total numbers of bronchitics represents prevalence of “obstructive bronchitis”. During the period from 1967 thru 1970 this prevalence was maintained at a higher level exceeding 50% although after 1971 it was less than 40% and demonstrated consistent tendency to decrease. The prevalence of cases with “bronchiolitis” was 14.0% during the period of 1967 and 68, showing tendency of gradual increase to reach 34.8% during the period of 1975 and 76. The trend to decrease prevalence of obstructive bronchitis seems presumably to be related to the improvement of environmental SO₂ pollution.

In 1975 we conducted pulmonary function survey in the Downtown Tokyo and in the suburban Islands area. We have chosen at randomly adult male subjects who were accompanied by persistent cough and shortness of breath. Num-

TABLE 1
Recent trends in bronchitics. The table indicates prevalence of “obstructive bronchitis” and “bronchiolitis” in the period of 1967 to 1976

	'67, '68	'69, '70	'71, '72	'73, '74	'75, '76
Total bronchitics	57	124	198	223	184
Abnormal FEV ₁ %					
Total bronchitics	58.2	54.6	30.3	38.1	32.6
Abnormal AaD and normal spiro:					
Total bronchitics	14.0	21.0	23.7	31.8	34.8

bers of bronchitics was 104 for the Downtown subjects and 72 for the Islands subjects. Among them numbers of patients demonstrated central airway obstruction were 33 (31.7%) for the Downtown area and 18 (25.0%) for the Islands area.

On the same group of subjects flow-volume curve was tested. Numbers of cases, who demonstrated normal ventilatory capacity with abnormally small V_{25} , were 36 in the Downtown area and 14 in the Islands area. Thus prevalence of abnormal flow-volume characteristics accompanied by normal spirometry was found to be 34.6% in the Downtown area while it was 19.4% in the Islands area. The prevalence of "bronchiolitis" in the Downtown area was consistently higher than that in the Islands area. We obtained the similar results on the arterial blood gas study. Taking $AaDO_2$ as the parameter we assessed the numbers of cases who were assumingly associated with distal lung damage without any detectable central airway obstruction and found prevalence of "bronchiolitis" to be 39.4% in the Downtown area and of 15.3% in the Islands area.

4. Local air pollution accidents

We had opportunity to study several local air pollution accidents. An example was the accident occurred on girl students of a junior high school located nearby a trash burning plant. A group of girl students, who were playing on the playground, suddenly started to complain difficulty of breathing, tightness of chest or chest pain, nausea and headache. Because of various circumstantial conditions this accident was finally assumed to be an air pollution accident although on this particular case we were not able to identify the pollutant to cause this

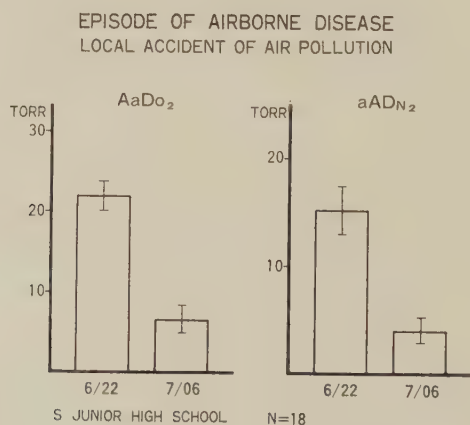


FIG. 3. $AaDO_2$ and $aADN_2$ obtained on a local accident of air pollution.

accident. We studied 18 students who demonstrated the overmentioned symptoms and found tachypnea, tachycardia, small bubbling rales as the common findings. None of the subjects demonstrated abnormally large 3Hz respiratory impedance measured by forced oscillation technique. We found on the day of the accident arterial hypoxemia with slight respiratory alkalosis accompanied by remarkable transient increase in $AaDO_2$ of 22.6 torr and in $aADN_2$ of 15.5 torr. Clinical findings and data on respiration physiology found on these students were similar to the “bronchiolitis” although the course of illness was acute and transient.

On accidents of oxidant exposure we found similar findings in respiration physiology, which were characterized by abnormally large $AaDO_2$ and $aADN_2$ with arterial hypoxemia accompanied by respiratory alkalosis.

Fig. 4 indicates the dose-response relationship in terms of $AaDO_2$ established on the oxidant-exposed subjects. Data were obtained on the arterial blood gas

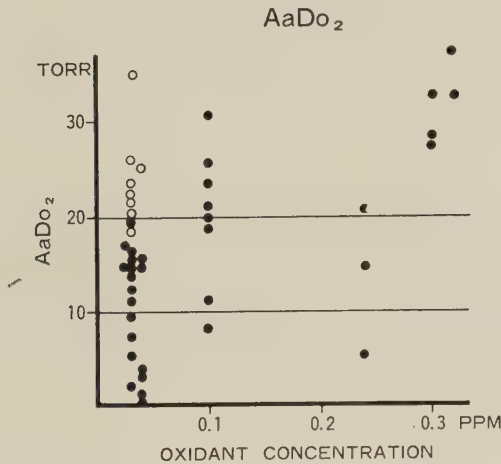


FIG. 4. Dose-response relationships obtained on the oxidant-exposed subjects. The open-circles indicate the subjects who were suffering from bronchitis when they were exposed to oxidant.

study performed within a few hours after exposure. In the figure the open circles indicated the subjects who were suffering from bronchitis when they were exposed to oxidant. Although the scatter of the data was rather significant, it seemed quite conceivable that the positive dose-response relationship was demonstrated. We further obtained similar results of dose-response relationship on oxidant-exposed subjects in terms of $aADN_2$

CONCLUSION

I have discussed the distal airway obstruction and/or alveolar damage as one of the possible airborne pulmonary diseases. I have focused my discussion on the distal lung damage, which was detected in terms of impaired alveolar gas exchange mainly due to ventilation-perfusion ratio unevenness.

I have introduced our criteria for "bronchiolitis" on the clinical basis to represent this distal lung damage. The "bronchiolitis" does not directly imply to the pathologically defined classical "Bronchiditis obliterans", which was proposed by Lange in 1901. According to our findings on the open chest lung biopsy, the transbronchial lung biopsy and/or on the selective alveolobronchography done on some cases with "bronchiolitis" it was assumed that our "bronchiolitis" and "Bronchiditis obliterans (Lange)" seemed to have close relation. Relatively high and increasing prevalence of this "bronchiolitis" was assumed to be presumably related to air pollution.

In conclusion I should like to conclude the paper again by placing my emphasis upon increasing significance of the distal airway obstruction to take over the classical central airway obstruction in the chronic obstructive lung diseases.

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Fibrosing alveolitis due to inhaled particles

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DEFINITION-NOSOLOGY

The phrase *fibrosing alveolitis due to inhaled particles* is meant to indicate a disease situation which takes place at the bronchiolo-alveolar level in relation to the inhalation of inorganic particles, with the consequent development of an inflammatory process involving principally the connective tissue of the alveolar septa, and which then usually evolves towards diffuse interstitial fibrosis.

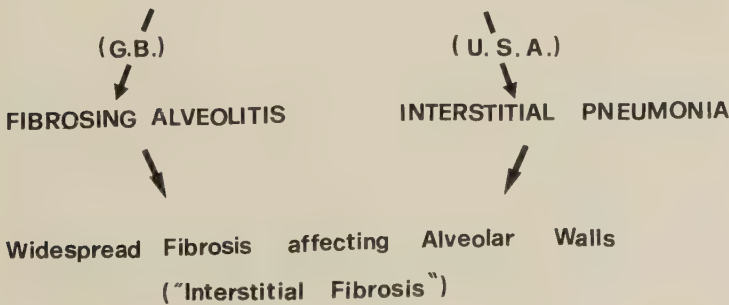
The *fibrosing alveolitis* of the English authors corresponds to the *interstitial pneumonia* of the American authors, (Table 1). It is distinguishable from those phenomena of *intra-and peri-alveolar exudate formation*, (due for example to bacterial infection), which cause the formation and organization of sero-fibrinous endo- and peri-alveolar exudate and disease processes with massive, localized fibrosis.

It is also distinguishable from localized or diffuse types of fibrosis which become established in *granulomatous disease* of varying etiopathogenetic derivation, which develops within the alveolar or bronchiolo-alveolar wall.

The "terminal" of these various situations is always a picture of *interstitial fibrosis*, which term was extensively used in recent years, following the first

TABLE 1

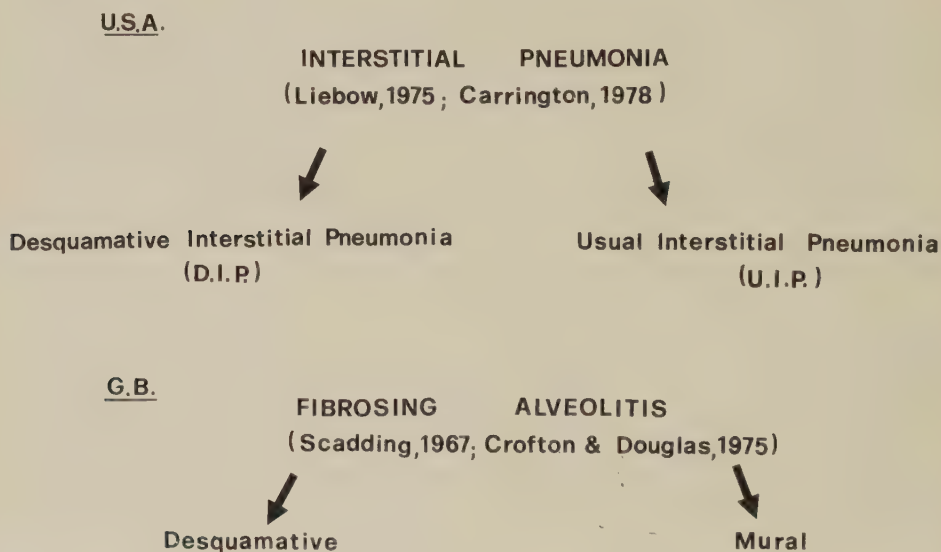
Inflammatory Changes in Supporting Structures of the Lung



descriptions by Hamman and Rich between 1941 and 1944, but which, nowadays, is only used for the final, irreversible and undifferentiated phases of the original process.

In the field of *fibrosing alveolitis-interstitial pneumonia* the American authors Liebow (1975) and Carrington (1978) distinguish between *desquamative interstitial pneumonia* (D.I.P.) and *usual interstitial pneumonia* (U.I.P.). They consider these to be two completely different entities from the point of view of pathogenesis (Table 2).

TABLE 2



D.I.P. includes those forms characterized histologically by the presence of large mononuclear cells on the alveolar surface and in the septa, by the relatively limited increase in thickness of the septal walls, and by the absence of fibrinous exudate or hyaline membrane. A characteristic of D.I.P. is its good response to treatment with corticosteroids. Its pathogenesis seems to be due to a single cause, although this is as yet unknown.

In U.I.P. on the other hand, there is usually a greater intraseptal involvement based on cell movements with a rapid evolution of fibrillogenesis. There is usually a poor response to corticosteroids. There seem to be various different pathogenic agents.

The English authors Scadding (1967) and Crystal (1976) use a similar terminology and differentiate *fibrosing alveolitis* into *desquamative* and *mural* va-

rieties, which are analogous to D.I.P. and U.I.P. The relative intermediate forms are probably related to the different etiologic agents responsible for the interstitial inflammation.

ETIOLOGY-PATHOGENESIS

There are many types of inorganic particle present in the atmosphere at home and at work which can penetrate the respiratory tract to reach the bronchiolo-alveolar tissues.

Among the inhaled inorganic substances some accumulate in the lungs without giving rise to any tissue reaction; typical examples of these are particles of carbon, tin and iron (Table 3).

TABLE 3

DISORDERS RELATED TO INORGANIC PARTICULATE INHALANTS

1. Non-fibrogenic:

coal, tin, iron

2. Fibrogenic:

silica, asbestos, talc and occasionally coal

(M.TURNER-WARWICK, 1975)

Others directly cause fibrillogenesis, or an inflammatory septal reaction which rapidly leads to the development of diffuse interstitial fibrosis. In this respect the actions of silica powder, asbestos fibres, talc, and sometimes of carbon, (especially when there are notable quantities of silica present together with the carbon), have been well known for a long time.

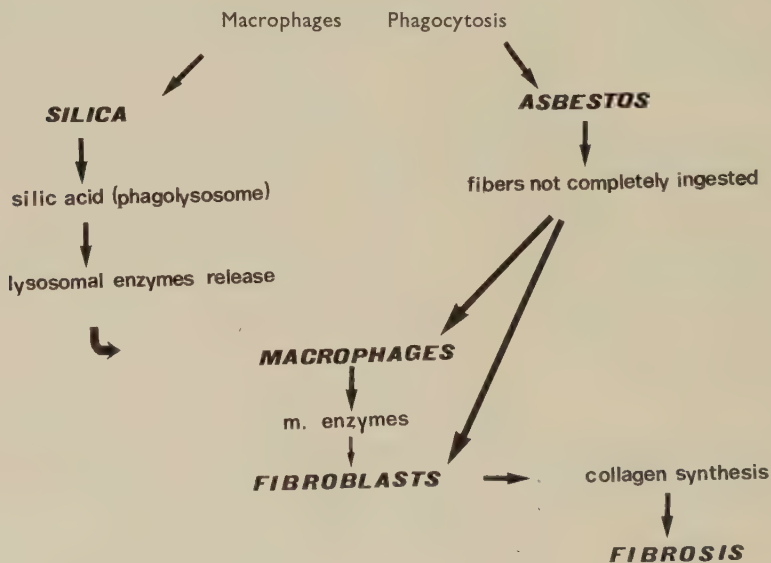
The *pathogenetic mechanisms* by which some inhaled particles, although passively deposited in large quantities within the alveoli, do not stimulate the biosynthesis of collagen, whilst others, such as silica and asbestos, clearly have a pathogenetic effect, are not yet known.

At the present time it is believed that liberation of certain macrophage enzymes is the stimulus which acts on the fibroblasts of the septal framework and

makes them synthesize collagen (Table 4). By means of preliminary hydroxylation of proline and lysine these enzymes are capable of forming the endoplasmic reticulum of protropocollagen, thence tropocollagen, and by integrating cross-linkages lead to the formation of fibrils.

In *silicosis* the silica particles are phagocytosed by alveolar macrophages. Following phagocytosis the silica reacts with water, principally within the phagolysosome, giving rise to silicic acid.

TABLE 4



Since the hydroxyl groups of silicic acid can act as hydrogen donors, they can alter the phospholipid structure of the lysosomal membrane. Damage to this membrane provokes liberation of macrophage enzymes followed by destruction of the macrophages themselves, even in the extra-cellular environment of the septal connective tissue.

Macrophage enzyme stimulation of fibroblasts is probably the main cause of the abnormal synthesis and deposition of collagen fibres in the alveolar connective tissue and of the consequent diffuse interstitial fibrosis.

In *asbestosis* it has been shown that the fibrillogenic effect is linked to the length and the diameter of the fibres rather than to their chemical composition, (which, on the other hand, is of importance for the effects of cell damage and tumour induction caused by asbestos fibres).

It is believed that the action of asbestos fibres takes place by a mechanism similar to that for silica, with mechanical damage to the alveolar macrophages and consequent liberation of enzymes into the septal connective tissue. Since the fibres are not completely phagocytosed by the macrophages, due to their greater length, they might also be having a direct effect on the fibroblasts.

PATHOLOGICAL FEATURES

The preliminary phase of fibrosing alveolitis due to particles such as silica and asbestos is composed of a lively proliferation of septal fibroblasts. These show a limited capacity to move from the septal margins and to desquamate within the alveolar cavity. This predominantly cellular phase is followed by a fibrillar phase with formation of precollagen and collagen along the alveolar walls, and gaining greatest consistency at the nodal points of the septal network.

In *silicosis* fibrosing alveolitis takes place on a large scale in the upper regions of the lung fields, with the frequent appearance of several small particles of silicon dioxide. These may easily be found outside certain arterioles and venules involved in the formation of nodules which are destined later to undergo hyalinization. Pleural involvement is relatively rare, consisting of a limited region of connectival changes.

In asbestosis cell migration and fibrillogenesis follow a similar pattern, but chiefly within the lower sectors of the lung fields. Fine needle-shaped particles are often found within the septal walls. Pleural involvement is conspicuous, with dense thickenings. Lymph node involvement is much less constant.

Septal fibrosis due to talc particles undergoes a similar course.

CLINICAL MANIFESTATIONS

Some clinical phenomena, such as crepitations, drum-stick fingers, dyspnoea, and alterations of gas exchange, are not considered to be diagnostic, since they are also found in idiopathic primary fibrosis.

The important distinguishing characteristics of fibrosing forms, with respect to the non-fibrosing forms and to primary pulmonary fibrosis (P.P.F.) are shown in Table 5.

With respect to *pulmonary involvement*, in anthracosis the lung regions most affected are the upper lobes and the lymph nodes of the trachea and the large bronchi. This is probably due to the relative hypoventilation of the upper lobes, and also to the process of migration of macrophages loaded with carbon pigment, from the peripheral lymphatics towards the hilar lymph nodes. In *silicosis* too, involvement is chiefly of the upper lobes. In *asbestosis*, on the other hand, the

TABLE 5

	INORGANIC PARTICULATE INHALANTS			CRIOPTOGENETIC
	NON-FIBROGENIC	FIBROGENIC		FIBROSING
	COAL	SILICA	ASBESTOS	ALVEOLITIS
PULMONARY INVOLVEMENT	Upper lobes, hilar lymphonodes	Upper lobes	Lower lobes	Basal or total
PLEURAL INVOLVEMENT	Rarely	Rarely	Frequently	Rarely
FIBROSING ALVEOLITIS	In some cases with ANA & RF	In some alveolar septa, well separated by normal alveoli	Associated with alveolar wall thickening and destruction	Destruction until widespread honey-combing

lower lobes are affected. In *P.P.F.* lung involvement is principally basal, or spread throughout the parenchyma.

Pleral involvement is rare in *anthracosis*, *silicosis* and *P.P.F.*, but is common in *asbestosis*.

With regard to the *orientation toward fibrosis* it is of interest to stress that in certain forms of *anthracosis* there is the appearance of septal fibrosis. This can readily be understood when it is realized that together with the carbon particles a noteable amount of silica is also inhaled. According to some authors, however, an important part is also played by the type of reaction of each individual. The serum of patients who develop fibrosis has been shown to contain anti-nuclear antibodies and to be positive for Rheumatoid factor with a greater frequency than in patients suffering only with pneumoconiosis.

In *silicosis* septal fibrosis typically takes place only in certain parenchymal sectors, whilst other alveolar zones are completely free of disease.

In *asbestosis* the septal reaction is always accompanied by marked thickening of the wall, with destruction of septae and changes in blood perfusion.

These alterations are analagous to those seen in *P.P.F.* which usually lead to total derangement of the lung structure, leading to the formation of "honey-comb lung".

Extrinsic allergic alveolitis

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Farmer's lung, bird-fancier's lung and related diseases fall under the generic term of extrinsic allergic alveolitis or bronchiolo-alveolitis (12, 23, 26, 28, 29). This is the disease which Ramazzini (1713) described under "Diseases of Sifters and Measurers of Grain" and which led them to "heap a thousand curses on their calling". They represent the responses of mainly non-atopic subjects to the inhalation of extrinsic agents capable of penetrating to and being retained in the peripheral gas-exchanging bronchiolo-alveolar tissues of the lungs. The many agents recognised as causes at present are mainly of organic origin, such as spores of actinomycetes and fungi and organic dusts. The possibility of inhaled chemical dusts or fumes being responsible as well cannot be excluded, though there is limited evidence of this.

CLINICAL FEATURES

The clinical features of farmer's lung, the classical example, or of bird fancier's lung manifest themselves either acutely, that is within several hours where exposure is intermittent or insidiously where it is regular and often small in amount (23, 26). Both forms of presentation may occur in the same subject, being determined by the circumstances of exposure.

The systemic manifestations consist of episodes of malaise, myalgia, fever and in many cases there is a rapid and considerable loss of weight together with a leucocytosis. The pulmonary features are dyspnoea which may be very severe and usually without wheezing, together with cough and on auscultation fine hair-like crepitant rales may be heard. Radiographic examination usually shows nodular or micronodular shadowing affecting the upper lobes particularly and being followed by severe fibrosis and cystic changes of the upper lobes if the diseases persists as a result of continuing exposure (26).

Physiological tests show a restrictive ventilatory defect with impairment in gas transfer as shown by the fall in the carbon-monoxide gas-transfer factor (DLCO). These findings contrast with asthma in which an obstructive ventilatory disturbance is present. The pathological changes are characteristic, consisting of usually patchy infiltration of alveolar walls and to a lesser extent of the respiratory bronchioles with lymphoid, plasma and histiocytic cells, together with

epithelioid cell granulomata and giant cells with cytoplasmic clefts (8, 11, 26). The clinical and other features of the acute reactions tend to resolve on cessation of exposure.

MAIN CAUSAL FACTORS

These can be divided into groups according to the nature of the causal agent, though there is no evidence so far that this influences the form of the disease. They include the following:

A. *Heterologous proteins*

The inhalation of foreign serum and other proteins occurs when these are in the form of dusts, for example: avian proteins from the droppings, which contain excreted serum proteins on drying (28, 17, 13, 14, 9); bovine and porcine proteins from posterior pituitary powders used as an inhaled source of vasopressin for the treatment of diabetes insipidus (25, 20); rat serum proteins which can be present in large amounts in the urine. It is probable that there are other similar or related sources of foreign proteins in forms which can be inhaled (26).

B. *Actinomycete spores from mainly thermophilic actinomycetes*

These are usually small, from 1 to 3 μ diameter and thus well suited for penetration to and retention on the alveoli. These come from many different sources: e.g. *Micropolyspora faeni* and *Thermoactinomyces vulgaris* from overheated mouldy hay and vegetable produce in farmer's lung (24, 12); *T. sacchari* from mouldy sugar cane bagasse causing bagassosis (29, 18). Spores from a number of different genera and species of actinomycetes have been identified in the dusts from hot-air and other ventilation systems associated with the production of extrinsic allergic alveolitis, so-called ventilation pneumonitis (15, 21, 27).

C. *Fungal spores*

Many different, otherwise innocuous, fungal spores have been identified as causes of disease. These include, for example, *Cryptostroma corticale* from mouldy maple-bark causing maple-bark stripper's disease (26); *Penicillium frequentans* from mouldy oak (cork) bark causing suberosis (1); *P. roqueforti* on cheese causing chesee-washer's lung (7); *Merulius lacrymans* (dry rot), *Pullularia pullulans* and *Graphium* on mouldy redwood dust (5); *Aspergillus clavatus* and also *A. fumigatus* on mouldy barley causing malt-worker's lung (4).

D. *Wood dusts* (30)

Saw-dusts from a variety of woods have been incriminated including Western Red Cedar.

E. *Mites*

Cheese-mites, mainly *Acarus siro* have been identified as another cause of cheese-maker's lung and these and other mites are probably more relevant to allergic alveolitis than has yet been established.

F. *Cereal and other dusts*

Fish-meal workers may also get allergic alveolitis from the inhaled dust.

G. *Humidifier lung*

Aerosols or mists of contaminated water can produce allergic alveolitis and whilst many fungi and bacteria are present in the water and antibodies shown against them, the main cause appears to be free-living amoebae of the *Acanthamoeba* genus, e.g. *A. castellani*, *A. polyphaga* (12, 27, 31).

H. *Miscellaneous causes*

There are a number of these including *Sitophilus granarius*, the wheat weevil (19); dust from the thatch of huts in New Guinea (2); and probably paprika-dust causing paprika splitter's lung.

IMMUNOPATHOLOGICAL MECHANISMS

The inhalation of the organic antigen leads to sensitisation as shown by the production of precipitating antibodies (23). With sensitisation, very small, sometimes minute, amounts of antigen suffice to elicit reactions and episodes of tissue damaging disease. The appearance of the reaction and of systemic and pulmonary manifestations several hours after acute exposure and its typical duration of 18 to 24 hours and sensitivity to corticosteroids are all features compatible with a serum-sickness like reaction in which Type III allergy mediated by toxic, soluble, complement fixing complexes are present. This is supported where the antigens are suitable for skin testing by the elicitation of Types I and III reactions. There is some controversy as to whether this mechanism is responsible alone, if at all. Whatever the case the precipitin test and other similar or related tests have been the means of identifying causal agents. Part of the reason for doubt is the presence of precipitins in exposed but apparently unaffected subjects (16). This is essentially correct in the first instance because the antibo-

dies are evidence of exposure and need to be associated with the appropriate clinical features to be of diagnostic significance. There are unexplained differences in the correlations of precipitins with disease. Thus, for example, many poultry breeders have precipitins to hen antigens but allergic alveolitis is uncommon, though it is by no means uncommon in those who rear poultry in the ordinary way and not in batteries; many pigeon breeders have precipitins and many have disease; by contrast budgerigar or parrot fanciers with precipitins almost always have disease. There is much cross-reactivity between the serum proteins of different avian species, though this is least so with the immunoglobulins of the birds' serum proteins and there is suggestive evidence that these may be more important as the responsible avian antigen related to the relevant bird species (13, 14). Recent agglutination tests using avian erythrocytes (9), which are nucleated and settle rapidly, have shown that the avian antigen on their surface which reacts with the avian antibodies is the light chain of the bird's immunoglobulins. A point of some interest is that antibodies to avian antigens in bird fanciers may be a source of false positive complement fixation tests with viral antigens raised in hens' eggs. Since many viral diseases have systemic and some pulmonary features as well, the above finding needs to be given consideration in interpreting results of "viral" antibody tests.

The presence of granulomata is suggestive of Type IV, lymphocyte mediated allergic reactions, supported by *in vitro* reactions of the lymphocytes to specific challenge, though there is as yet no evidence of the full picture of Type IV allergy, as shown in the delayed skin test reaction. Granuloma formation in which sensitised lymphocytes are involved may be induced by particulate antigens (3). Insoluble antigen-antibody complexes can also elicit granulomatous reactions. There are thus several possible mechanisms operating independently or together in their production (26).

Complement activation by the alternative pathway without the participation of antibody has been shown with a number of the causes of allergic alveolitis (22, 10). This may explain, and probably contributes to, some episodes of disease but seems unlikely as the explanation for the repeated attacks which can be elicited by very small doses of the causal agent. The activated complement in turn activates macrophages which may contribute to antigen processing and subsequent antibody production and also to granuloma formation.

CONCLUSIONS

Inhaled organic dusts produce different diseases according to the immunological reactivity of the subject. Atopic subjects tend to produce IgE antibody in response to very limited exposures and develop immediate type asthmatic

reactions. Non-atopic subjects tend to produce precipitating antibodies in response to more intensive exposures, capable of mediating Type III reactions and resulting in extrinsic non-immediate asthma or extrinsic allergic alveolitis. Agricultural environments in particular provide exposures likely to cause these respiratory diseases.

The initiation of the disease in subjects who may have been exposed for many years without apparent effect, may arise from re-exposure after a period of cessation of exposure. The history should be taken with particular attention to this possibility as it can lead to identification of the causal agent and to a correct diagnosis, failing which exposure is likely to continue with severe life-threatening consequences.

The mode of presentation of the attacks is also influenced by the circumstances of exposure with acute attacks after intermittent exposure. The relationship between exposure and disease is thus made more readily evident. Where the exposure is regular the disease develops more insidiously, the relationship to the cause is less readily evident and usually apparently obscure, so that the disease tends to be more severe.

It is thus clear that all inhaled antigens should be regarded with suspicion in subjects with obscure fibrosing lung disease or even recurrent pyrexial episodes of unknown origin. Diagnosis of cryptogenic pulmonary fibrosis (diffuse interstitial pulmonary fibrosis) should only be made after full consideration has been given to extrinsic allergic alveolitis.

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Chronobiologic methods for medical prevention from air pollution in city areas

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To prevent air pollution in city areas nature, origin and amount of pollutants must be known as well as the predictable variability of this latter as a function of time. In addition, any one of these items must be considered from technical, economical, social and ethical points of view. One has to realize that a city is a complex organism in which the high level of inter-individual and group relations favors and stimulates the production of goods (and the related technical, intellectual and cultural means) develops competition and creates needs. The major source of air pollution is the energy consumption (Ramade, 1974; Turk *et al.*, 1974). It is of interest to know that technological methods to bring air pollution under control are already available (Turk *et al.*, 1974; Gervais, 1976).

Contrasting with both the advance and the efficiency in means able to reduce air pollution, medical problems have not retain the attention they deserve. Particularly, the variability in the risk of exposures to air pollution is not usually taken into account. With chronobiologic methods, it is now possible, on the one hand, to consider circadian (about 24 h) and circannual (about 1 year) changes in the susceptibility of human beings to environmental factors (Smolensky *et al.*, 1972; Reinberg and Halberg, 1971) as well as to take opportunity of the fact that asthmatic subjects play the role of biologic detectors of air pollution (Reinberg *et al.*, 1971; Gervais and Reinberg, 1977; Halberg *et al.*, 1977). Therefore, this type of patients can be regarded as sentinel organisms. Both interindividual differences and predictable group variability as function of time of the day and time of the year can be quantified for a better estimation of the risk for a population as a whole.

Thus aims of present report are:

1. to summarize some answers to the question: how, technologically, air pollution can be prevented (or reduced)? Related sociological, economical and political problems are beyond the scope of this paper;

2. to show by selected examples that chronobiological methods can be useful to evaluate the tolerance of exposure to air pollution in both individuals and group of subjects.

Obviously, these two approaches are complementary; e.g. reactions of sentinel organisms can be used for a better understanding of levels under which air pollution must be maintained.

Technological prevention of air pollution

A city is an open system. A large variety of substances (including matters providing energy) are imported into the city (e.g. gasoline, fuel, food, etc.) and thereafter used and transformed. A certain amount of substances are exported out side the city as refuse or waste. However, a large amount of waste (resulting mainly from the consumption of energetic substances) is discharged into atmosphere and remains more or less in the city as air pollutants.

Among *major gaseous pollutants* one finds: Carbon monoxide (CO); compounds that contain sulfur (sulfur dioxide: SO_2 ; Sulfurtrioxide: SO_3); compounds that contain nitrogen (Nitrogen oxide: NO; nitrogen dioxide: NO_2 or both grouped as NO_x); hydrocarbons; "Oxygenates"; Ozone (O_3) and oxidants, hydrogen fluoride (HF).

Among *particulate air pollutants* (airborn particle matter) one finds: *viable particles* (pollen grains, spores bacteria fungi, molds, insect parts, etc.); natural materials (sand and soil particles); *manmade particulate pollutants*: the smoke and the soot both resulting from the incomplete combustion of coal, oil, wood, garbage and herbs (including cigarette, cigar and pipe smoke which is an important form of indoor air pollution). It has to be emphasized that all sooty smokes contains carcinogenetic agents. *Organic and inorganic particles*: (insecticides, lead used in gasoline), *metallic and nonmetallic mineral products*.

According to Turk *et al.* (1974) and Gervais (1976) the air pollution can be controled by several specific and unspecific methods. It is possible to protect individuals (gaz mask?) or houses. (At the Rothschild Foundation in Paris we are using since 1968 allergen -and particle—shielded rooms to explore allergic patients). It is possible to reduce air pollution either in using less—polluting sources of energy (e.g. solar energy is non-polluting) or simply in using *less* energy.

To control air pollution at the source two main classes of methods can be considered: 1) the pollutants are separated from the harmless gases in order not to be discharged into the air; 2) the pollutants are somehow converted to innocuous substances that may be then discharged.

Devices such as bag filter and mechanical shaking, cyclone collector, electro-

static precipitators, tall chimney etc. have proved to be efficient and are already used on a large scale. Even for automobiles using gasoline (they are one of major source of air pollution in city areas — CO; NO₂; oxygenates and hydrocarbons—) effective anti-pollution devices have been developed.

Conventional air quality standards are obsolete

Whatever methods of air pollution control may be proposed and applied reference to an index of “tolerable” concentration of pollutants in the air must be made. Various air quality standards have been published and used. (e.g. air quality standards set by the U.S. Environmental Protection Agency, the New York City Department of Air Resources and the WHO). There is no doubt that their existence is an important step forward. However, the pollution index selected for these standards are a kind of compromise between what is conventionally needed and what is economically feasible.

From a chronobiological point of view we believe that these air quality standards are obsolete for the following reasons:

1. The susceptibility of the human organism to potentially noxious agents (including air pollutants) is not constant as implicitly postulated. In fact, it varies predictably, as function of the time of the day and as function of the time of the year.
2. Levels of air pollution themselves vary predictably in both a 24 h scale and 1 year scale; this fact is underestimated.
3. New objective criteria, such as circadian changes in the bronchial patency of asthmatic patients, must be considered to suggest what are actually means and endpoints of a desirable index.

Circadian and circannual changes in the human susceptibility to potentially noxious agents

A large number of experiments have shown that animals (including man) exhibit both circadian and circannual changes in their tolerance to physical and chemical noxious agent to which they are exposed (see review papers by Reinberg and Halberg 1971; Halberg, 1969; Reinberg, 1976; Scheving *et al.*, 1974).

Stupfel *et al.* (1977) have demonstrated in rats that CO is more toxic during the dark span (when animals are active) than during the light span. Furthermore, breathing 480 ppm CO during dark span is about as much toxic as breathing 1700 pp, CO during the light span.

Statistically significant circadian changes have been demonstrated in the reactivity of allergic subjects to a set of substances including allergens. This

is true for the skin reaction to intradermal injections of histamine, house dust extract, penicillin, pollen and feather extracts (Reinberg *et al.*, 1969; McGovern *et al.*, 1977), as well as for the bronchoconstriction resulting from inhalation of histamine (Tammeling *et al.*, 1977), acetylcholine (Reinberg *et al.*, 1971) and house dust (Gervais *et al.*, 1977). Figure 1 shows circadian changes in the bron-

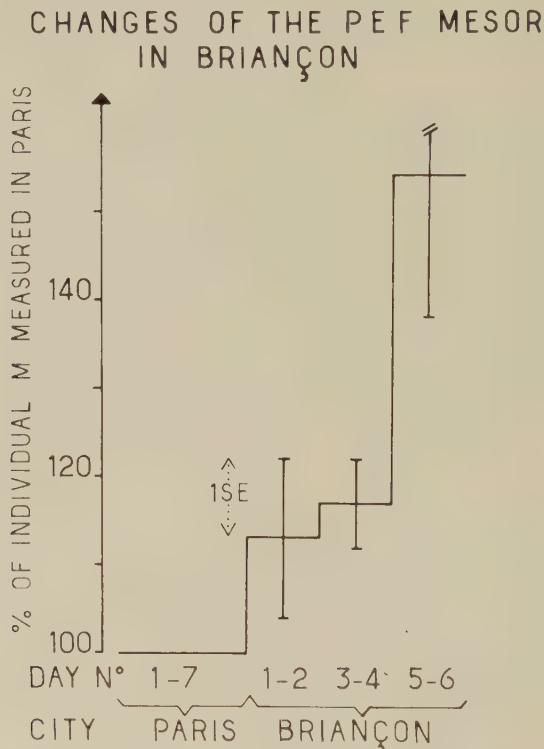


FIG. 1. Changes in the bronchial patency (FEV_{1s}) as a function of the timing of a bronchial challenge to house dust extract. Tests were performed every other day at four different clock hours with dose fixed for each asthmatic. Results are expressed as percent of fall with respect to pretest time point FEV_{1s} values. The largest fall ($22 \pm 5\%$) corresponds to inhalation of the house dust at 11 p.m. (23.00) while the smallest ($7.5 \pm 2\%$) occurs for the test at 3 p.m. (15.00). The difference is statistically different ($p < 0.025$).

chial patency (FEV_1) as a function of the timing of bronchial challenge to house dust extract in aerosol.

Let us underline that the chronotoxicity (or the chronotolerance) is predominantly if not exclusively endogenous (Reinberg and Halberg, 1971; Halberg, 1969; Scheving *et al.*, 1974).

Circannual changes in morbidity and mortality resulting from lung diseases have been demonstrated in USA (Smolensky *et al.*, 1972) as well as in France (Reinberg *et al.*, 1973) Table 1. The circannual acrophases (peak times) occur in winter months with a great stability in time (from decade to decade) as well as in space (from state to state). Regional differences in acrophases remain small between e.g. California, Minnesota, Illinois, Texas, New York and France. Such results suggest that human beings may be more sensitive, during winter months, to air pollutants such as SO₂, NO_x, bacteries, virus, etc. In turn these circannual changes in susceptibility may be related to circannual changes in hormone secretions (Reinberg and Lagoguey, 1978) and immunological processes (Reinberg and Schuller *et al.*, 1977) among others.

TABLE 1
Circannual rhythms of orbidity and mortality (cosino summary)

<i>Diseases</i>	<i>Monthly M or (No. of cases)</i>	<i>Amplitude A* in % of M (95% confidence interval)</i>	<i>Acrophase Ø Ø ref. Dec. 21st</i>	<i>Ref.</i>
Respiratory				
Morbidity	(7.4×10 ³)	55 (22 to 89)	26 Nov. (7 Oct. to 22 March)	**
Mortality	(4.2×10 ³)	53 (39 to 68)	30 Janv. (7 Jan. to 11 Feb.)	**
Cardiovascular				
Morbidity	(5.0×10 ⁴)	18 (9 to 27)	21 Jan. (13 Nov. to 20 Feb.)	**
Mortality	(4.2×10 ³)	16.5 (12.5 to 20.5)	7 Feb. (24 Jan. to 20 Feb.)	**
Mortality	3346± 75	16.3 (12.2 to 20.4)	23 Feb. (2 Feb. to 13 March)	***
Stroke	5387±133	19.6 (12.7 to 26.5)	20 Feb. (9 Feb. to 2 March)	***

* Amplitude = 1/2 of the total variability; ** USA: Smolensky M.H., Halberg F., Sargent F. (1972); *** France: Reinberg A., Gervais P., Halberg F. *et al.* (1973).

Circadian and circannual changes in air pollutant production

Air pollution varies according to: 1) human needs and activities and 2) meteorologic factors (adiabatic lapse rate, atmospheric stagnation, atmospheric inversion, etc.). Our activities and needs are periodic and thus predictable: e.g. production of heat during cold months, diurnal work and nocturnal respose. Air pollution resulting mainly from energy consumption, continuous recording of air pollution levels shows rhythmic changes in both the 24-hour and the 1 year scales.

For instance SO₂ levels recorded in Paris (1966-1971) reached their annual peak in December-January (257 to 326 µg/m3) and their annual trough in Au-

gust (32 to 48 $\mu\text{g}/\text{m}^3$). The SO_2 peak in the 24 hour scale occurs at ~ 17.00 (5 p.m.) and can be as high as 1515 $\mu\text{g}/\text{m}^3$.

Again in Paris, the traffic-related CO pollution exhibits two peaks, the first one between 8-10 a.m., the second and the larger one between 4-6 p.m.

Contrasting with these predictable circadian and circannual changes, variations of air pollution levels due to meteorologic factors appear to be randomly distributed during fall and winter.

Let us assume that results obtained by Stupfel *et al.* (1977) on rats can be extended to men. Chronotoxic effects of CO will reach their peak in the early afternoon, precisely at a time of day corresponding to high levels of CO in streets and in tunnels.

From a chronobiologic point of view air quality standards can be improved in taking into consideration both the cyclic variations of air pollution and the cyclic variations of the human susceptibility to noxious agents. In other words, air quality standards must not be limited to average values but presented as daily and yearly curves (e.g. sine function).

Asthmatic patients are sentinel organisms

Asthmatic patients appear to be highly sensitive to air pollution (Charpin, 1973, 1976; Zweiman *et al.*, 1972; Carnow *et al.*, 1970; Oshima *et al.*, 1969). Let us underline that the bronchoconstriction (associated with a measurable fall in the bronchial patency) occurs not only when the asthmatic patient inhales the allergen(s) to which he is specifically sensitized (e.g. house dust, insect parts, feathers, pollens) but also when both chemically or physically irritating agents reach human airways and bronchiae. Asthmatic patients represent 2 to 6% of populations of industrial areas.

Portable instruments such as peak flow meters and spirometers can be given to an asthmatic patient for self-measuring (e.g.; 5 times a day a fixed clock hours) his vital capacity and/or his peak expiratory (flow PEF). Thus it is possible to quantify circadian changes in the bronchial patency (with reference to PEF) of this patient when exposed to different levels of air pollution. Circadian changes can be quantified by several parameters the acrophase (peak time), the amplitude and the 24-h rhythm average or mesor M. Only the latter parameter is here considered.

As result of several chronobiological experiments, the bronchial patency of patients suffering from allergic asthma (as reflected by PEF circadian mesor) has been shown (with statistically significant differences) to improve in clean air.

Sojourn of adults patients in allergen and particle-shielded rooms (Reinberg *et al.*, 1970; Gervais *et al.*, 1977).

— Sojourn of children in heavy (Paris) and low (Briançon 1300 m, French Alps) polluted areas (Fig. 2) (Gervais *et al.*, 1978).

— Sojourn in a rural small village of a chromium-plating worker suffering from allergic asthma when working in Paris (Table 2) (Gervais *et al.*, 1978).

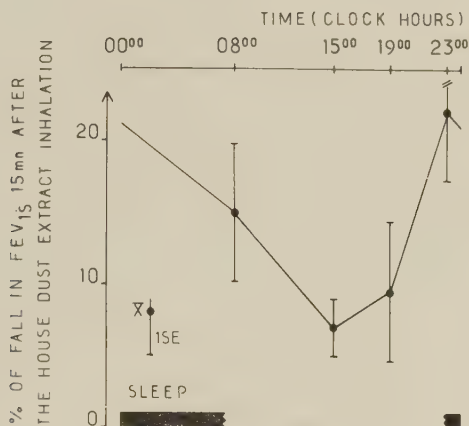


FIG. 2. Five children living in Paris (3 girls: 9 to 11 years; 2 boys: 8 and 11 years), suffering from allergic asthma (house-dust) documented time series of peak expiratory flow. Self-measurements were performed, under medical control, 5 times a day at fixed clock hours (10.00, 13.00, 16.00, 19.00, 21.00) during a 14 days span, at least. Data were collected first in Paris for 7 days and immediately thereafter in Briançon again for 7 days.

The auto-rhythmometric method was used to quantify objectively variations of the bronchial patency resulting from exposures to air pollution this latter being relatively high in Paris and low in Briançon (French Alps 1300 m/sea level).

Individual mesor values of PEF (means of 7 days) were higher in Briançon than in Paris, the difference was statistically significant (not presented). For the group considered together, the PEF mesor showed a progressive rise from day n. 1+2 to day n. 5+6 in Briançon ($p < 0.025$).

Even for non-asthmatic subjects such statistically significant differences can be demonstrated (Halberg *et al.*, 1977). A 17 years old woman non-smoker, without lung allergy but exhibiting allergic responses of skin to copper, nickel, chromium and perfumed cosmetics performed PEF self-measurements in Paris (29 days) in Minneapolis (72 days) and again in Paris (46 days). PEF mesor in metropolitan Paris was 360.1 ± 0.9 (1 SE) l/min; it improved in suburban Minneapolis reaching 373.7 ± 0.6 l/min and decreased to initial values when back in Paris.

Such findings lead to the conclusion that some human subjects (e.g. patients suffering from allergic asthma) can be considered as sentinel organisms or biologic detectors of overall air-pollution. Easy-to-do and not expansive chrono-

TABLE 2

Autorhythmometry Method. Changes in the PEF Mesor Related to the Exposure to CrO₃ of a Chromium-Plating Worker

<i>Situation</i>	<i>Autorhythmometry Month</i>	<i>(No. of days)</i>	<i>PEF circadian mesor in l/min ± 1 SE</i>	<i>P</i>
Work 1	July	(5)	394 ± 20	< .02
Work 2	July	(5)	396 ± 22	< .05
Vacation 3	August	(9)	464 ± 15	—
Work 4	September	(7)	394 ± 18	< .01

Man (38 years) working in a chromium-plating work-room (4 years); suffering from dyspnea and/or asthma attacks (3 years). Skin tests and bronchial challenge to Cr were not conclusive. PEF self-measurements were done at fixed clock hours (08.00, 11.00, 15.00, 19.00 and 23.00) during 5 to 9 days, both when attending the workroom during the day (situation 1, 2 and 4) and when in vacation (situation 3) in a rural area. With reference to values obtained in the latter situation, a statistically significant fall in the PEF circadian M occurred when this man was exposed to CrO₃.

PEF circadian acrophases were located around 15.00 for all the considered situations. PEF circadian amplitudes were ~9% of M.

biological methods can be used to objectively quantify the reactivity of these sentinel organisms to air pollution. In addition, the air pollution-related risk can be estimated individually by the same methods as it was the case for the chromium-plating worker. Data thus gathered can be integrated with other items used to construct new air quality standards.

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SYMPOSIA
GEOGRAPHICAL PATHOLOGY

Geographical considerations in internal medicine

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Even now, some two and a half millenia after Hippocrates' teaching, mankind is still indebted to him for the basic insight which underlies all approaches to geographical pathology today. In "Airs, Waters and Places" he drew attention to environmental risks of several sorts. Man is unavoidably subject to the hazards of his climate, either sweltering hot or frigid, and to other inescapable factors of his physical surroundings such as soil conditions and wind chill. In contrast Man has certain options about his life-style. He may choose loose-living and lechery or he may choose a Spartan way-of-life. In his recreational pursuits too he exercises choice; maybe to smoke cigarettes, to climb mountains or to hunt lions. The geographer's task is to assess all of these forms of hazard to health from the totality of human environment insofar as communities may be affected as distinct from the clinician's care primarily for the individual sufferer.

The group of papers in this Symposium is drawn from widely different physical and social environments. Their point of common interest lies in concentrating attention upon surroundings and place because the geographic contribution to public health centres upon environmental detective work leading to recognition, and hopefully amelioration, of culpable factors exogenous to Man.

Naturally many methods of study will be needed but three approaches especially dominate the field of geographic pathology and each of the papers to follow falls broadly into one of these categories.

The first type of study is definitional where the over-riding and simple purpose is to identify the places and patterns of risk. For example, Figure 1 illustrates a seven-year series of individual male deaths from a group of alcohol-related causes by place of home residence (McGlashan, 1978). Moreover by comparison with local populations-at-risk, corrected for age, one can say that three areas, including the capital city Hobart, have significantly more than their fair share of these deaths whilst some northern localities exhibit significantly few male alcohol-related deaths. From such a map social causes may be hypothesised and preventive action planned.

The second approach depends upon the comparison of a disease pattern with one or more environmental factors. These may relate to the disease directly

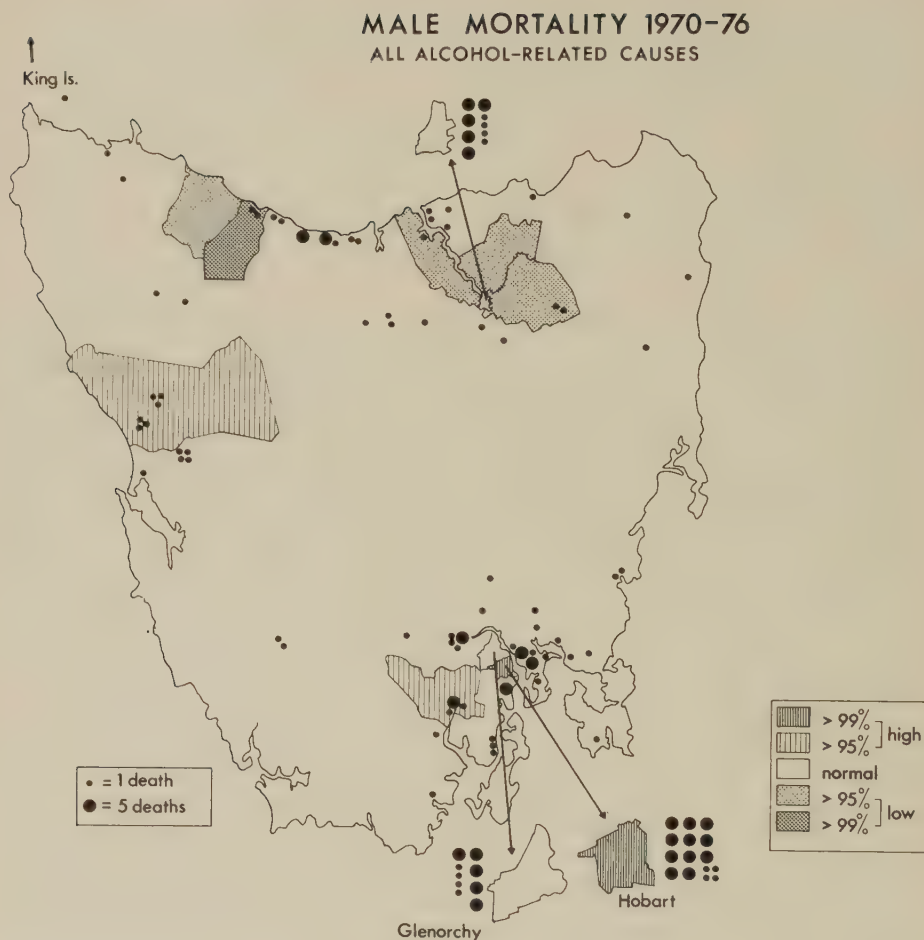


FIG. 1

by influencing the biogeographic habitat of virus or vector. In Figure 2 Bentley (1916) shows the influence of malaria upon the growth or decline of population in East Bengal. Again the aim is to further understanding of associative relationships for use in public health programmes.

Thirdly spatial aspects may be considered with the additional dimension of time. Often ill-health data can be displayed as a series of "still" pictures in instant time. A sequence of stills run consecutively develops a cine-record of disease movement over time. Where repetitive and distinctive diffusion routes emerge, as in Figure 3 portraying infective hepatitis spread by three distinctive

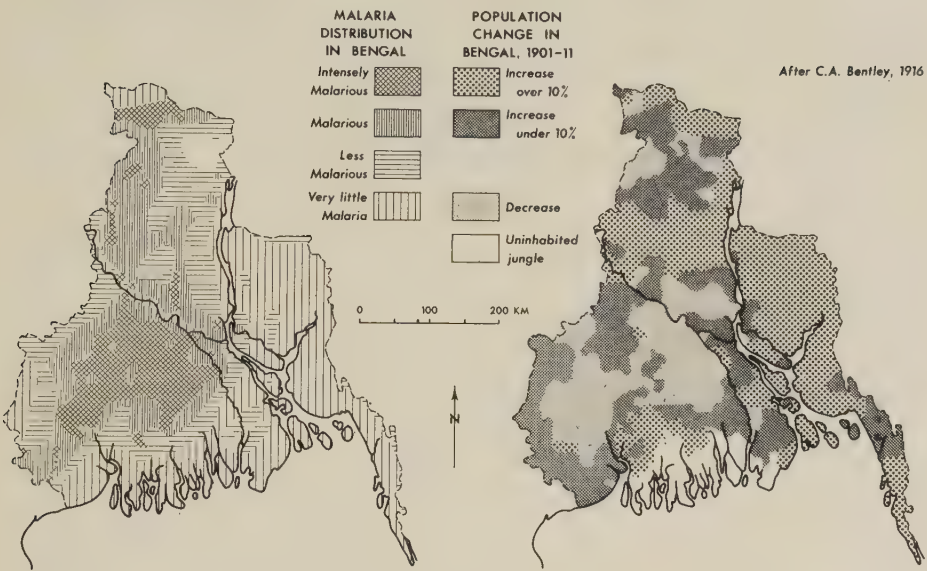


FIG. 2

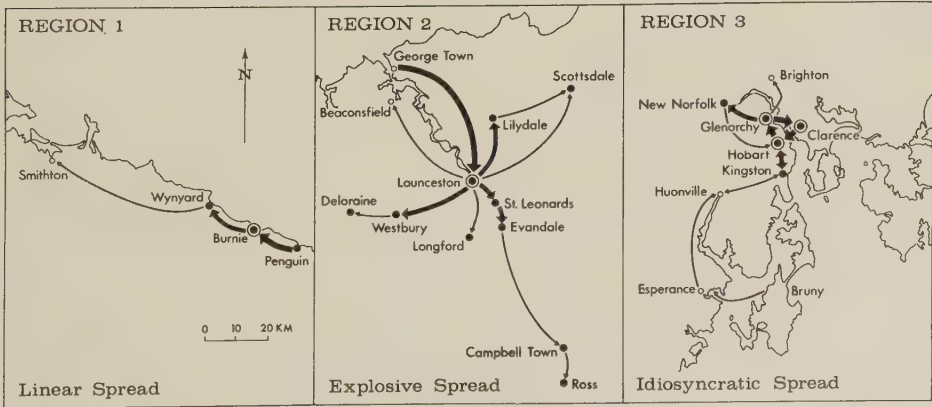


FIG. 3

types of pattern in Tasmania (McGlashan, 1977), then the modes of spread must be sought in order to set up prophylactic barriers to such disease movement. Quarantine measures against cholera-bearing migrants into north America are an example of the reality of such measures in the past (Pyle, 1969).

The most critical geographic vision is that disease occurrences vary over space and reflect the fact that human health depends dominantly upon lifestyle and total environment.

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Tropical eosinophilia (T. E.)

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Tropical eosinophilia has been reported from many areas including India, the Philipines, Indonesia, Malaysia, Australia, Latin America, China, North Vietnam and South Vietnam. From Hong Kong and Philipines, it is reported that the disease is very rare there. So also is the case with Japan. There is, however, no reliable data available from any of the South East Asian region regarding the prevalence rate of this disease. It has, however, been found that the disease is definitely more common in those countries where filariasis is endemic. Therefore, it appears that the geographic distribution of filariasis may be related to the geographic distribution of tropical eosinophilia.

So far as India is concerned, which no doubt is a vast sub-continent, we have reliable information regarding the prevalence of filarial infection in different States of the country. Even though no actual data are available regarding the prevalence rate for tropical eosinophilia in the general population, I have been able to get information regarding hospital admissions for tropical eosinophilia, and I have been able to calculate the number of admissions for the disease per thousand of total admissions for all diseases. Such data, in respect of different States alongwith prevalence rate for microfilarial infection are indicated in the following map.

It is of interest to note that in those States like Rajasthan, Punjab, Himachal Pradesh, Jammu & Kashmir, where filarial infection is extremely rare, no reports of admissions for tropical eosinophilia have been received. A study of geographic pathology of the disease has, therefore, provided additional evidence in favour of the etiologic hypothesis that filarial infection is related to the development of tropical eosinophilia.

There are no doubt other circumstantial evidence to show that filarial infection in some form or other, is related to the development of tropical eosinophilia. Positive complement fixation test with *dirofilaria immitis* antigen (Danraj *et al.*, 1957), production of tropical eosinophilia symptoms in a human volunteer by injecting animal filarial larvae by Buckley (1958), and finding microfilarial sections in lung biopsy by Web *et al.* (1960) are some of the evidence in favour of filarial aetiology.

Finding of antibodies against human microfilaria and filarial larvae in all cases of elephantiasis tested and in majority of cases of tropical eosinophilia by

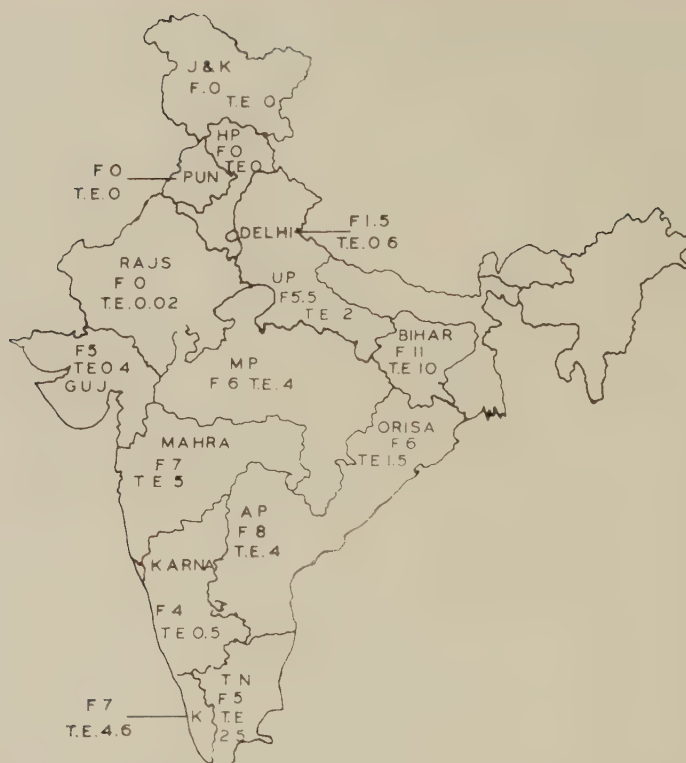


FIG. 1. Map showing admission rates for T.E. and filarial infection.

Wong and Guest (1969), is in favour of human filarial infection as the basis for development of tropical eosinophilia syndrome. Our own finding of positive leucocyte adhesion test with *microfilaria bancrofti* significantly more than with *D. immitis*, is in favour of *M. bancrofti* being the cause of T.E. Increase of IgM and IgB in both elephantiasis and tropical eosinophilia more or less to the same extent, go to show that this disease is possibly an immunological reaction to human filarial infection and the same immune reaction is the cause of disappearance of *microfilaria* in the peripheral blood of elephantiasis patients and those suffering from tropical eosinophilia (Viswanathan *et al.*, 1973 and Viswanathan, 1973).

Study of geographic pathology has now provided further evidence in favour of human filarial infection since the geographic distribution of tropical eosinophilia coincides with that of human filarial infection in different parts of India. Hence if tropical eosinophilia is filarial in origin, human filaria is more likely to be the cause than animal filaria.

We had the opportunity to study the natural history of the disease in a large series of 1051 patients with tropical eosinophilia during a period of 5 years in an area where filariasis is endemic and one in every chronic respiratory patients is a case of tropical eosinophilia. There were 62.3% males and 37.7% females. 23.5% were in the age group 0-14. It is of interest to note that it included 3 infants, below 1 year. 32.8% were in the age group of 15 to 29. 43.7% were 30 years and above.

Admissions for tropical eosinophilia for the years 1972 to 1976 in the V.P. Chest Institute were analysed and age specific admission rates were calculated. In the age group 0-4, admission for males was 1.9% while for females it was 3.2%. In the age group 5-14, it was 4.3% and 2.4% respectively, and in the age group 15 and above, it was 3.4% and 1.7%. This shows very clearly that there is no significant difference in the age specific admission rates for tropical eosinophilia. From this, one can infer that all age groups are more or less equally affected and there is no significant difference in the prevalence rate by sex.

Analysis of the data on the basis of duration of illness at the time of admission showed that 30.6% of patients had the disease for 1-6 months, 7.3% for 6-12 months, 37.3% for 12-160 months and 24.7% for 60 months and over.

490 patients had sudden onset of the disease while in the remaining 571, the onset was insidious. This only shows that if routine blood examinations is done on all the patients attending a Chest Clinic, it should be possible to detect cases of acute onset, is most common in T.E.

In the analysis of cases with acute onset, 8.8% started with influenza fever, 18.6% with acute naso-bronchial allergy and 19.3% with sudden asthmatic paroxysms.

Insidious onset was characterised by hacking cough with exertional dyspnoea in 43% of the cases and hacking cough with paroxysmal dyspnoea in 29% in the whole series. Onset of fever was observed in 43.3% and haemotysis in 6% of the cases.

57.8% had rhonchi in lungs and 61.8% had rales. Liver was enlarged in 1% and localised lymphadenopathy was seen in 1.5%. 34.8% of the patients had absolute eosinophil counts between 2000 and 5000. In 27.9%, it was 5 to 10 thousand while in 37.3% it was over 10,000.

Study of a number of biopsy specimens and review of published reports suggest the following possible pathological evolution of the disease. The disease starts as eosinophilic interstitial pneumonitis, which later may cause eosinophilic exudation into alveoli. At a later state, localised granuloma with foreign body type giant cells are formed. Some of the untreated cases may develop interstitial fibrosis which can lead to increased pulmonary vascular resistance and cor pulmonale.

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Environmental determinants of California encephalitis in Ohio

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Since the early 1960's, when the reporting of arthropod-borne encephalitis was substantially improved in the United States, it has been possible to accumulate knowledge about contributory environmental risk factors. When viewing the entire country, for example, evidence indicates that Arbovira encephalitis has a high probability of occurrence in areas drained by the Mississippi River and major tributaries (1). Figure 1 contains reported cases of encephalitis per 1,000,000 persons in 1975 (2). About half of the cases shown were confirmed as Arboviral encephalitis. Parts of the country with higher reporting also generally correspond with the Mississippi migratory bird "flyway".

Arthropod-Borne Encephalitis

In geographical pathology it is common practice at the discovery of many disease-causing agents to name particular diseases after locations where they have initially been isolated. One kind of mosquito-borne encephalitis, now widely accepted as California encephalitis, is known to be part of a larger inter-related group of viruses affecting humans. Venezuelan, West Nile, Simliki Forest, Bunyamwera, Ntaya, Japanese B, Russian Spring-Summer, Eastern, Western, St. Louis, LaCrosse and California are all names which have been used. In different parts of the world it is expected that different names would be used for variations of arthropod-borne encephalitis characterized by similar symptoms in humans. Clearly, not all species of mosquitoes are widespread throughout the world. Still, important aspects of arthropod-borne encephalitis include generally similar environmental conditions which can be compared.

Given knowledge of geographic factors contributing to the disease, it can also be controlled by limiting the proliferation of particular kinds of mosquitoes in areas identified endemic to arthropod-borne encephalitis (3). Methodological developments within the past 25 years in medical geography and medical knowledge about arthropod-borne encephalitis can be combined to study the disease problem. The following four steps can be utilized in accomplishing such a task: 1) the identification of biogeographic aspects of mosquito-borne encephalitis

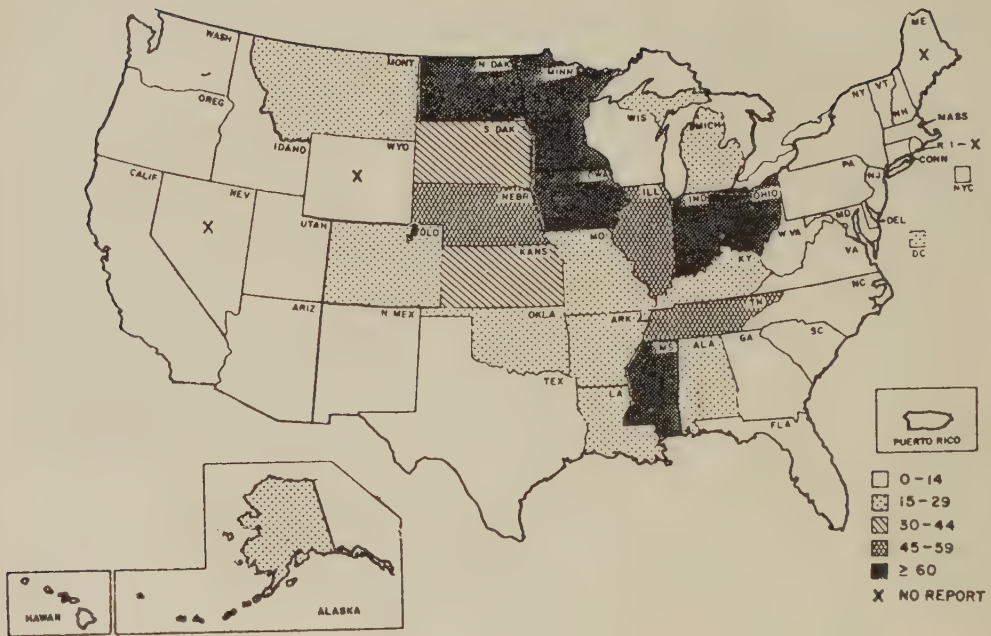


FIG. 1. Encephalitis in the United States in 1975.

common to diverse locations; 2) the study of common aspects of environments which appear to be contributing to excessive amounts of arthropod-borne encephalitis; 3) on the basis of generalized mapping develop more detailed scale identifications of endemic areas; and 4) identify specific environmental risk hazards within endemic areas and compare these areas for evidence of common environmental problems.

For a period of approximately 30 years (1933 to 1964), unique yet related viruses were investigated within a variety of locations in the United States and other parts of the world. Areas known to have encephalitis problems were selected as locations for the establishment of laboratories. The identification of what has become known as California encephalitis was first published in substantial detail in the early 1950's, although information had been gradually accumulating since the early 1930's (4). Hammond, Reeves and Sauter reported the isolation of encephalitis virus from *Aedes dorsalis* and *Culex tarsalis* mosquitoes in their now well-known 1952 study of the San Joaquin Valley of California (5). Laboratory techniques included the use of small mammals, domestic fowl, horses and wild birds for evidence of encephalitis virus isolated in mosquitoes and humans. It was concluded that a variety of intermediate hosts could be

identified, particularly the mosquito-wild bird-mosquito virus life cycle and to some extent small mammals as intermediate hosts. Eventually, epidemiological researchers were to make the statement that arthropod-borne encephalitis was "widespread geographically" (6).

During the 1950's and early 1960's, studies of arthropod-borne encephalitis virus increased. A related virus was recovered from a Snowshoe hare in Montana in 1955, and in 1958 a similar virus was recovered in Czechoslovakia and termed Tahyna (7). It was evident within the United States by the mid-1960's that a complex interrelationship between several kinds of encephalitis virus, for example, LaCrosse and California, recurred continuously in serilogic studies within different parts of the country. By 1964 a complex California encephalitis group was well known (8). Epidemiological studies had associated the disease generally with environments akin to more natural habitats and to farm areas. In many instances horses and barnyard fowl were identified as having similar encephalitis characteristics. Wild birds and small mammals normally inhabiting temperate forests were tested and found to be immune carriers. Combinations of such environmental conditions as presence of woodlands, areas with poor drainage, and places with periodic cycles of rainfall were considered to be somehow contributing to outbreaks of California encephalitis in humans.

During the several decades of identification of California encephalitis few cases were reported either to state departments of health or to the Communicable Disease Center in Atlanta, Georgia. After 1964, however, when the California group was better known, reporting increased. Of particular importance was knowledge of the relationship between the LaCrosse virus first identified in Wisconsin and the general California group. The LaCrosse strain was subsequently identified in horses in a semi-rural part of southeastern Pennsylvania, and in 1964 several kinds of mosquitoes were discovered to be carriers of California encephalitis in Ohio (9). One particular location in Ohio, the village of Gambier, was considered to be endemic; and detailed epidemiological field studies of the area attributed the high rates of California encephalitis to several environmental phenomena. Mosquito proliferation was explained by the presence of a mixed mesophytic forest including a particular kind of Silver Maple that seemed to attract mosquitoes. It was also implied that glaciation of the area contributed to poor drainage conditions allowing for many locations where mosquitoes could breed. Also, it was noted that farm animals were found in the area immediately surrounding the small village. Figure 2 is adapted from an epidemiological field map of the Gambier area constructed by Dr. Richard Berry and his colleagues investigating the encephalitis problem. The black dots show the distribution of human California encephalitis cases from 1964 to 1971 in Knox County. Note the number of cases in areas of mixed forest.

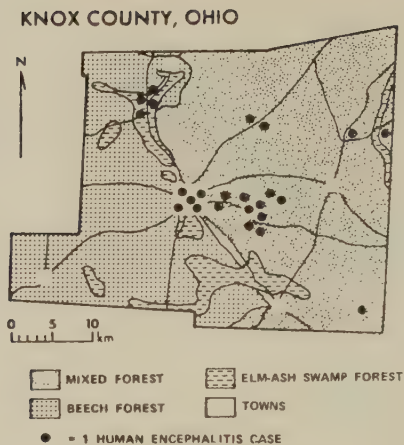


FIG. 2

The epidemiological field map constructed by Bardos and Danielova in Czechoslovakia more than a decade earlier shows some interesting environmental similarities (Fig. 3). Bardos and Danielova selected a study area in the southern portion of East Slovakia; a lowland area known to have a high mosquito population. There were farms in the area and numerous rivers contributed to periodic flooding and the development of poorly drained areas. The assumption was made that circulation of the virus among wild and domestic animals could

TAHYNA AREA OF CZECHOSLOVAKIA

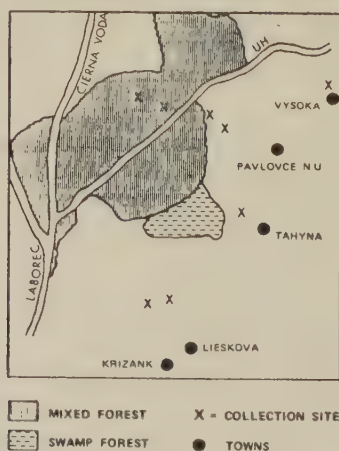


FIG. 3

be identified in relation to the geography of the area. In addition, this part of East Slovakia is in the path of migratory birds possibly contributing to wintering-over of the virus. An encephalitis virus was identified from mosquitoes trapped near the village of Tahyna. Although large numbers of mosquitoes were tested in relation to the number of positive isolations, the most success was achieved when examining mosquitoes found in farm sheds. The important aspect of this study was that encephalitis virus was identified in *Aedes caspius*, a kind of mosquito closely related to *Aedes dorsalis*, one species later known as an encephalitis carrier in the United States.

Comparing Figures 2 and 3, several questions might be posed pertaining to what these areas have in common. One aspect that stands out in both studies is the information about excessive mosquito populations due to poor drainage and proximity to mixed forests. Following this environmental 'clue', it is possible to examine associations in more detail. Cases of confirmed California encephalitis reported in the state of Ohio from 1964 to 1975 seem to cluster in areas within and adjacent to floodplains (Fig. 4). The large cluster between Columbus and Mansfield is in the Know County area. When a comparison was made between the temporal distribution of cases of confirmed California encephalitis and rainfall in endemic areas, it was discovered that disease outbreaks follow summer rains. Figure 5 shows the temporal distribution of confirmed cases from 1964 to 1975. Normally there is early reporting in the spring and a gradual increase during the months of June, July and August with a peak in September. Reporting declines with decreasing temperatures. When a comparison is made with rainfall peaks, a lag ranging from one to two weeks can be identified between the time that rainfall peaks and encephalitis reporting increases. This complex environmental association consists of California encephalitis endemicity within parts of the state, accumulation of water following rain in poorly drained areas adjacent to the epidemic areas, the increased breeding of mosquitoes, and the transmission of California encephalitis to humans. Intermediate hosts, such as wild or domestic mammals or birds, are probably involved in this transmission cycle.

A detailed examination of the distribution of some confirmed cases of California encephalitis within the Akron, Ohio area (considered endemic) explains how natural environmental factors also contribute to arthropod-borne encephalitis in an urban industrialized area. The Akron area is on a drainage divide between waters flowing into Lake Erie and the Ohio River. Previous glaciation in the area caused substantial disruptions in former drainage patterns and the development of many glacial lakes and poorly drained areas. In the northwestern part of the city, the valley of the Cuyahoga River is fairly wide and deep. In addition, the floodplain of an older stream is contained within a larger valley.



FIG. 4. Encephalitis Prevalence and Floodplains in Ohio.

Parts of the valley are wooded and ravines draining into the Cuyahoga River Valley form portions of a metropolitan park. Figure 6 shows the distribution of confirmed cases of California encephalitis in relation to the ravines and wooded areas. Epidemiological field studies indicated that mosquitoes were no doubt breeding in the floodplain below the populated portion of this area. Somehow the California encephalitis virus was carried up the floodplain into the settled

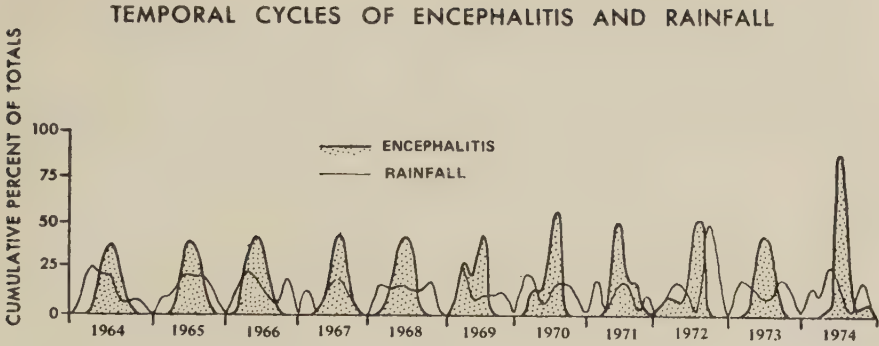


FIG. 5



FIG. 6

area to cause infection in children. Perhaps the mosquito-wild bird-mosquito association was involved. Perhaps wild mammals were involved. On the other hand, perhaps there was no intermediate host. The important point is that environmental factors contributing to the spread of California encephalitis were postulated. By selectively spraying the floodplain below the settled area and portions of the metropolitan park, the problem of California encephalitis was brought under control.

The disease still has epidemic potential, and control of the disease is a problem of timing. The most effective spraying programs are those implemented immediately after the peaks of rainfall. However, this peak cannot be determined in advance, and the best measures are to continue to spray in poorly drained

areas with floodplain-like characteristics. What is not known is how the disease winters over. For example, there is speculation that it might be maintained in domestic pigeons as well as small mammals. One possibility is that it winters over in warmer climates within the red-winged blackbird, known to be an encephalitis virus carrier. The virus could be annually returned to the state via these birds. This kind of transmission possibility is suggested as an area of further research in geographical pathology.

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The resurgence of malaria in India 1966-77

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In 1947 when India gained her independence from British rule it was estimated that there were about 80 million cases of malaria per annum, with many thousands of deaths. There were well studied regional differences in the prevalence of the disease, with a macro-regional contrast between endemic and epidemic India. The dominant parasite was *Plasmodium vivax* with a comparatively small but significant proportion of cases of *P. falciparum*. The principal vectors included: the very versatile *Anopheles culicifacies*, breeding in still water-bodies of all sizes and degrees of permanence from irrigation reservoirs to hood-prints and discarded cans; however, hyperendemic areas were particularly associated with mosquitoes of wet jungles, *A. fluviatilis* and *A. minimus* breeding in clear running forest streams (except during inundations during the wet season). In West Bengal *A. philippinensis* was regarded as preferring still water but avoiding a very high water table, while along the coasts *A. sundanicus*, a brackish water mosquito of coastal lagoons, was responsible for wandering epidemics. In towns and cities in western India, including Bombay, *A. stephensi* has become well adapted to urban life, breeding in wells in city courtyards, in more modern water cisterns and similar sites, and this adaptation was recorded as well established by about 1925. Lastly in the north-west, now in Pakistan, *A. superpictus* announced a transition to the Middle East realm in anopheline species and other biological phenomena.

From about 1950 onwards, malaria control campaigns mainly using DDT and other residual insecticides against mainly house-haunting anophelines proved so effective that in 1956 the target was changed from control to eradication. By 1965 it seemed that eradication was almost in sight, with only about 100,000 cases and no deaths recorded. Unfortunately that year proved to be the trough year, so far as can be seen at present, and since then the number of cases has increased almost uniformly, doubling or trebling in most years, and also extending over larger and larger territories in most years (Akhtar and Learmonth, 1977).

The territorial expansion can be seen as a diffusion from four residual foci resistant to the campaigns, in Kutch, Madhya Pradesh, the Orissa highlands and in highland Assam. In Kutch the vector was the urban mosquito *A. stephensi*, the others probably exophilic forest mosquitoes like *A. fluviatilis* and *A. minimus*.

The appearance of a diffusion from these four foci can be appreciated from a very quick and cumulative view of annual parasite index maps. For purposes of this paper I shall not pause to examine detail year by year and region by region, nor to point out the very few and comparatively slight retractions of the area of spread of the disease.

This pattern apparently of rediffusion of the disease has been accompanied by changes in the parasite. *P. vivax* remains the dominant species, but there has been an increase in *P. falciparum* in a belt across middle India. Moreover there are reports of resistance to chloroquine and other drugs in Assam particularly, so quinine has become more important again, and indeed emergency stocks are being built up in other areas in case drugresistance spreads.

A. culicifacies remains the dominant vector, but *A. stephensi*, the urban vector, has increased its territory along the coasts of peninsular India. Both species are developing considerable resistance to insecticides, as in *A. fluviatilis*, one of the classic forest vectors. In Assam the forest mosquitoes have been shown to have contributed to the persistence of malaria through exophily in pockets; a forest vector, *A. balabacensis* has assumed a fresh importance following the failure of the eradication campaign.

The younger but senior author of this paper, being the one based in India, will attempt to throw light on the resurgence through collating field work. However, that task is formidable. After all there are some 50,000 malaria control workers in the country, so it will be impossible to have more than a fraction accompanied by an entomologist, ecologist, population or migration geographer or the like, or even to have observations specially recorded in a consistent way for their analysis. Moreover there will be particular difficulties in throwing light on the problems raised by this apparent diffusion pattern retrospectively across the thirteen years since the resurgence began. It is for these reasons that we have decided that we must attempt a simulation of the diffusion. Our likely collaborator in this research, Dr. Gerald Pyle of Akron, Ohio, USA, is happily with us at this conference. We expect to build on the basic ideas on diffusion across space of Hägerstrand (1953, 1967). We shall return to Hägerstrand's ideas presently.

Simulation of an epidemic of malaria is not new to the literature (Macdonald, Cuellar and Foll, 1968). We follow earlier work by Macdonald, and a line of mathematical thinking dating back to Sir Ronald Ross, and it may well be to Grassi also. The essentials of the formula used for computer simulation of a specific epidemic in Katsina in Northern Nigeria are:

$$h = \frac{z_0 r x_{t-i} (-\log_e p)}{a x_{t-i} - \log_e p},$$

a = the average number of men bitten by 1 mosquito in 1 day;

r = the proportion of affected people, who have received 1 infective inoculum only, who revert to the unaffected state in 1 day;

t = time in days;

z_0 = the basic reproduction rate, or the number of infections distributed in a community as a direct result of the presence of a single, primary, non-immune case;

p = the probability of a mosquito surviving through 1 whole day;

h = the proportion of the population receiving infective inocula in 1 day;

x = the proportion of people affected (that is showing parasitaemia).

Ideally a study of this spatial pattern of resurgence should apply this formula to several hundred reference points across India. At this stage we do not think that this ideal research design is possible; we think that the data problems already noted close this avenue, at least at present. So let us return to Hägerstrand's ideas, or rather to an adaptation of them to the present problem as we see it.

Like many models used in analysis of spatial patterns, this one depends on initial assumption of a homogeneous space. If the "innovation"—in this case malaria—can be diffused by completely free and simultaneous communication from an initial occurrence to all other points in the space (in our case people), then random diffusion over the whole space would be appropriate. However, in the diffusion of information, rumour, innovations like telephones in rural areas, and many others, showed that this extreme assumption of homogeneity does not generally occur even in the most homogeneous areas open to study. Nearer points (or people) tend to be affected by the diffusion sooner than distant ones. So random diffusion can be simulated in successively widening belts Hägerstrand called these information fields, a term appropriate to his studies; we might call them host migration fields. If host migration fields are acceptable as a working concept, we should of course recall that human malaria has alternate hosts, man and *Anopheles*, so that migration of either host has to be envisaged. Hägerstrand appreciated very well that areas of any size can not be successfully modelled as if completely homogeneous. However, even in simulation it is possible to modify the chances of successful diffusion according to successive hypotheses about factors which encourage or discourage the spread of the innovation (or disease). This has been attempted with some success for infectious hepatitis in New South Wales (Brownlea, 1972). Given sufficiently detailed knowledge of movements of people and pathogens, and in our study of *Anopheles* also, the random element could of course be reduced and forecasting be made highly reliable so long as the patterns of movement and of immunity remained constant. Again our data problem is such that a strong random element is likely to pervade our studies through all the stages we can foresee.

Turning now to the resurgence of malaria in India since 1965, the pattern

we saw looks very like a diffusion from the four residual tracts which we have called Kutch, Madhya, Orissa and Assam. However, we shall have to check that *in situ* increases in malaria can be ruled out; as always there are going to be problems in working retrospectively. Preliminary calculations and historical precedents both suggest that some actual movement of hosts must be involved as well as *in situ* increase even where that seems to be predominant; however we shall examine *in situ* increase *pari passu* with each spatial simulation.

The successive simulations will produce figures for malaria control units or possibly for grid squares; we shall treat these as Expected figures, to be compared with the actual figures as Observed, and the significance of the null hypothesis tested.

The successive stages will be:

1. *In situ* increase.
2. Random diffusion across Indian space.
3. Random diffusion in successively wider host migration fields.
4. As 3), but with increased probability of success for the pathogen if population density is high.
5. As 4), but for low population density.
6. As 4), but for strong migration flow.
7. As 4), but for rapid urbanization (This could well be tested by straightforward statistical correlation, but we may find that urban malaria is indicated in some areas and not others, and this would be concealed by natural statistical analysis).
- 8a) As 4), but for wet years in dry areas (the alternative hypothesis of *in situ* increase may be especially important here).
- 8b. Increased chances downwind.
9. As 4), but for increased canal irrigation.
10. As 4), but for increased "tank" (small reservoir) irrigation.
11. As 4), but for increased well irrigation.
12. As 4), but for increased total irrigation.
13. We may be able to proceed to a simulation combining the influence of several factors, indicated as promising in explanation, from exercised 1-12.

CONCLUSION

We have demonstrated an apparently spatially coherent spatial diffusion pattern in the resurgence of malaria in India 1965-76. *In situ* increase from generally low endemicity rates in 1965 can not be ruled out at this stage, indeed will be tested *pari passu* with other hypotheses involving diffusion patterns. Random diffusion will be simulated within successively widening "host migration

fields" (following Hägerstrand's "information fields"); further simulations will build on this as basic, modifying in turn for high and for low population density; for new canal irrigation, tank irrigation, well irrigation, and total irrigation; for rapid urbanization; and perhaps for different combinations of 1-12.

It is claimed that spatial diffusion exercises may have a valuable role where, as in this instance, retrospective data problems are enormous and even present and future data collection may be extremely difficult. Macro-scale diffusion exercises may at least point the way to causal explanations appropriate to different parts of a large, populous and varied country of sub-continental scale. Micro-scale sampling may then be possible economically, in order to throw further light on causal explanations. These causal explanations will of course not be at the level of the basic malaria cycle, well understood for generations, but rather at the scale of how exactly the nearly successful malaria eradication campaigns of 1965 have been apparently defeated in a pattern apparently rolling across most of the country. Such explanations are likely to lie in elucidating different types of man-mosquito contact, and different types of human and anopheline behaviour in regionally differing patterns.

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Chagas' disease

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INTRODUCTION

Chagas' disease is an antroponosis common to man, to domestic and to wild vertebrates of the rural and periorban zones of the latin-american countries.

Oswaldo Cruz and Carlos Chagas share the discovery of the parasite which causes the infections: *Trypanosoma cruzi*, 1909. Chagas then published the first cases of human infections, a finding which was later followed by fascinating clinical, anatomo-pathological, epidemiological and laboratory studies which under an admirable scientific rigor were realized in the Manguinhos School by Villela, M. Torres, C. Pintos, E. Dias *et al.*

In spite of the enormous diffusion which *Trypanosoma cruzi* has among the rural population of America, this disease remained practically ignored until in Argentina in 1932 the portal of entry of the primo-infection into man was recognized. This did greatly help in the diagnosis of the acute forms. Later on, the xenodiagnosis and the diffusion of serologic methods allowed also the diagnosis of the latent and chronic forms of this disease.

PREVALENCY AND IMPORTANCE

T. cruzi infection extends from the south of the United States to the north of Patagonia in Argentina. For the latin-american countries it constitutes the major endemic sanitary problem, although in the different areas, the proportion is not the same. Epidemiologic researches have shown that many millions of human beings are infected. Fortunately the greater part of them are asymptomatic carriers of latent forms of the tripanosomic infection.

LIFE CYCLE AND PATHOLOGY

T. cruzi is a flagellate of which trypomastigotes forms are circulating in the blood of infected vertebrates. These forms penetrate into the interior of certain cell types (reticulo-endothelial-system, myocardial cells, brain cells, fat cells, liver cells). In the interior of these cells they transform into leishmania forms (amastigotes) and into crithidials (epimastigotes) by binary division; the latter then again turns into the infectious trypomastigote form. This cycle also takes place in the digestive system of the transmitter insect. The trypomastigote are

expulsed from the vector's rectum together with the feces. In the vertebrates, the multiplication of the *trypanosomes* inside the cells leads to the formation of pseudocysts which break up at the end of the evolution, a phenomenon that provokes the formation of a phagocytosis focus in which mobilized organ-cells ingest non-viable parasites and cellular debris. The inflammatory foci are the basis for the pathological features that characterize *Trypanosoma cruzi* (Romaña, 1942).

VECTORS, RESERVOIRS AND TRANSMISSION

The vectors of the *Trypanosoma cruzi* are haematophagous hemiptera that belong to the subfamily of *Triatominae*. There are some more than 100 species in this subfamily, of which the major part are sylvatic. A certain number has adapted to the living quarters of human beings, and these are the insects that transmit the parasitosis in domestic surroundings (especially of man, dogs and cats). The most adapted and most dangerous species are: *Triatoma infestans*, transmitters in Argentina, Uruguay, Paraguay, Chile, Bolivia, South of Brasil and South of Peru; *Rhodnius prolixus*, in the north of Brasil, Guayana, Venezuela, Colombia, Panamá and the Caribbean area of Central America; *Triatoma dimidiata* on the pacific coast of Ecuador, Colombia, Panama, Central America and Mexico. All the triatomide species are american with the exception of one, which is called; *Triatoma rubrofasciata* which, originally from Brasil, seems to have been taken to Africa and to the Far East by Portuguese ships. Some infections of man and monkeys by tripanosomas similar to cruzi, found in Malasia, have been attributed to this species, which today is cosmopolitan.

The sylvatic triatomidia species live in caves and dens of wild animals: opossum, foxes, squirrels, armadillos, rats, mice, etc. It is supposed that trypanosomosis cruzi develops in two parallel cycles, one domestic and the other sylvatic, cycles which rarely interchange the parasite.

The transmission of the infection generally occurs by contact with the dejections of the insect deposited on the mucus membrane or on the abraded skin.

There are also congenital infections transmitted from the mother to the fetus and, in endemic areas, transmissions by blood transfusions are very frequent today.

CLINICAL SIGNS: ACUTE, LATENT AND CHRONIC PHASES

The infection by *T. cruzi* presents itself in the human under three forms: acute, latent and chronic.

The acute form is generally found in children who are infected while sleeping in their homes. The infection goes along with irregular fever, tachicardia, generalized edema, meningoencephalic symptoms, hepato-splenomegaly, etc.

The diagnosis is easily oriented by the observation of the ocular portal of entry of the infection (sign of Romaña) with monocular edema and satellite lymph nodes; or the cutaneous inoculation chancre. The finding of *T. cruzi* in blood confirms the diagnosis.

The *asymptomatic forms* are the more frequent and generally pass inadvertently; they are only discovered by epidemiological researches or by routine serology.

The *chronic forms* may appear under a *cardiac symptomatology*: They are the most frequent and the most severe. They are characterized by extrasystolic arrhythmias, atrio-ventricular blocks, cardiomegaly, hypotension, etc., syndroms which generally end by sudden death or by chronically progressive asystolia. In certain countries and particularly in certain areas of Brasil the development of *megaviscera* (megaesophagus, megacolon, megaureter, etc.) have been attributed to the trypanosic infection. Although there exist a number of positive arguments in favour of this etiology, there is no consensus of criteria among researchers because there is no epidemiologic superposition between the Chagas' disease and the megavisceras. In Venezuela, for example, where *T. cruzi* infections are frequent, megavisceras are very rare.

It is difficult to prove the trypanosic etiology of the chronic *nervous forms* of the Chagas' disease (paresis, hemiplegia, paraplegia, mental disorders, etc.). The isolation of *T. cruzi* in patients from endemic areas is insufficient for vinculating the parasite with the syndrome. However, the nervous sequela in those persons who have outlived acute infections with meningoencephalitis furnish a strn basis for such a cause-effect relation.

LABORATORY DIAGNOSIS

During the acute period it is generally easy to find *T. cruzi* in the blood by examining freshly stained slides directly. In negative cases, xenodiagnosis will solve this problem.

During the *chronic period* xenodiagnosis or serologic tests have to be used: R.F.C.; I.F.I., Hemoagglutination, etc.).

TREATMENT

Until today all tentatives for curing this disease by trypanocides have failed. Some products, specially those derived from the nitrofuranes destroy the circulating trypomastigote forms in the blood but they do no act on the intracellular forms.

ENVIRONMENT AND CHAGAS' DISEASE

Chagas' disease is a sanitary problem directly related with the geography because until today it is a protozoonosis strictly American. It is curious that the other human trypanosomiasis, the sleeping sickness, is also restricted to a zone, which in this case is Africa.

The continental distribution of both infections is in relation with the transmitters who have a continental habitat: The triatomideos in America and the glossinas in Africa. Should it be that Chagas' disease could turn into a cosmopolitan infection through *Triatoma rubrofasciata*? It is difficult to predict that, but it is a problem that can be considered.

Chagas' disease is also related with the biology of the triatomidea and with the social, educational and economic surrounding of the populations in which it is developing. The miserable and primitive habitat (rancho, cañua, chabola, etc.) of the whole rural and periurban American zones are an ideal shelter for the triatomideos. They multiply in darkness where night by night they suck the blood of their crowded human and animal victims and contaminate them with their feces. The lack of education favours the indifference and the promiscuity with the insects and the low economic level prevents the improvement of the inhabitant's surrounding.

Some years ago the triatomidea developed only in the houses of rural areas, but when emigrating to the urban centers in search of better life conditions, the farmers have taken with their implements the insects or their eggs thus contaminating their new living quarters.

Today it is frequent to find autochthonous cases of Chagas' disease in the periphery of Buenos Aires or of Rio de Janeiro.

PROPHYLAXIS

The prophylaxis against trypanosomosis cruzi is based on three requirements:

1. Transformation of the miserable housings into adequate living quarters.
2. Well organized and efficient sanitary education.

3. Domestic desinestation which—apart from certain exceptions—should be done by the inhabitants themselves who should acquire consciousness of the danger that is surrounding them. The official domestic desinestation has failed so far in practice.

Epidemiology of sarcoidosis

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WORLDWIDE DISTRIBUTION

Sarcoidosis has a worldwide distribution but it is more frequently recognised in sophisticated communities. Whenever tuberculosis or leprosy are rampant, sarcoidosis will be in eclipse, but as they are brought under control so will sarcoidosis become more evident. Thus, worldwide epidemiological surveys should seek it in the wake of declining tuberculosis and leprosy. In Uruguay (1), the eradication of tuberculosis and the widespread use of mass miniature radiography brought to light a surprising frequency of sarcoidosis.

Data on 3676 patients in 11 cities—London, New York, Paris, Los Angeles, Tokyo, Reading, Lisbon, Edinburgh, Novi Sad, Naples and Geneva—revealed that the disorder is surprisingly similar in various parts of the world (2). There is no sex predilection and the patient usually presents in the 20-40 age group either because of respiratory symptoms, ocular or skin disease, or because of a routine chest radiograph. Sarcoidosis is multisystem with intrathoracic and reticulo-endothelial involvement, eyes and skin predominating (Table 1). The

TABLE 1

Percentage frequency of involvement of various tissue-systems in 3676 patients with sarcoidosis

<i>City</i>	<i>Intra-thoracic</i>	<i>Reticulo-* endothelial</i>	<i>Eyes</i>	<i>Skin</i>	<i>Erythema Nodosum</i>	<i>Parotid</i>	<i>Nervous system</i>	<i>Bone</i>
London	84	40	27	25	31	6	7	4
New York	92	55	20	19	11	8	4	9
Paris	94	32	11	12	7	6	4	4
Los Angeles	93	46	11	27	9	6	2	4
Tokyo	87	24	32	12	4	5	4	2
Reading	89	30	16	13	32	5	9	1
Lisbon	88	29	6	18	12	2	4	13
Edinburgh	94	39	11	7	33	5	3	1
Novi Sad	90	14	15	4	11	3	1	11
Naples	99	0	0	0.4	6	0	0	0
Geneva	97	17	12	6	11	2	1	3
TOTAL %	87	28	15	9	17	4	4	3

* Reticulo-endothelial = Spleen and peripheral lymph nodes.

Kveim-Siltzbach test was positive in 78%, the tuberculin skin test negative in 64%, hyperglobulinemia in 44% and hypercalcemia in 11%. Steroids are now being used in one-half of patients according to this survey. In this worldwide survey of huge series of patients, the mortality was about 2% due to sarcoidosis and 1% due to other causes.

NEGRO POPULATION

U.S.A. Much shoe leather has been expended on the epidemiology of sarcoidosis in the United States. It is evident that sarcoidosis is about ten times more frequent in the American negro than in the white population. Interestingly, the sarcoidosis profile is also different in the two groups. One-third of the black sarcoidosis patients compared with only 2% of the whites were born in the South Eastern United States. Female preponderance was only noted in the black patients. Respiratory symptoms, pulmonary infiltration, fever, weight loss, skin and eye lesions are significantly more common in blacks. Serum globulin elevations and eosinophilia are largely confined to black patients.

South Africa. The previously held view that sarcoidosis is rare in the black population of South Africa is no longer tenable. A recent survey at Groote Schuur Hospital, Cape Town (3) reveals an incidence per 100,000 of 17 in coloured, 27 in black and six in white patients. Only in black patients were skin lesions widespread and florid. This accounts for the mistaken diagnosis of leprosy in the past. Morrison (4) reported a series of 18 black sarcoidosis; all suffered from gross skin lesions, including lupus pernio, papules, nodules and plaques (some psoriasiform), a few lesions in the sites of old injury, and nail dystrophies. Two-thirds of the afflicted had intrathoracic involvement, one-half had bone cysts, and the eyes were involved in one-third of this series. What was of particular interest in this survey is that some of these patients had been mistakenly incarcerated in a leprosy institution. The diagnosis of sarcoidosis was only entertained when patients had failed to improve on antileprotic, antituberculous and antisiphilitic treatment. This reminds one of the bad old days when patients with sarcoidosis were also mistakenly detained in tuberculosis sanatoriums. In those days sarcoidosis was considered when patients failed to respond to antituberculous chemotherapy or when the tuberculin skin test was found to be negative.

EUROPE

Sarcoidosis is also frequent in the white population of Scandinavia and throughout Europe. It is most frequent in sophisticated diagnostic centres. In our London series of 818 patients the place of birth was the United Kingdom

in 74%, but the significant figures of 10% were from the Caribbean and 8% were from Ireland. In a mass chest radiography survey carried out in London, Brett (5) showed that the prevalence of intrathoracic sarcoidosis among ethnic groups was particularly high in Irish women and in West Indians (Table 2).

Just as London sees much sarcoidosis amongst its migrants from the British West Indies so does Paris see it in its Martiniques. Is this due to genetic predisposition or environmental factors confronting susceptible individuals migrating from a rural to urban community?

TABLE 2

Prevalence of intrathoracic sarcoidosis among ethnic groups in London (Brett, 1971)

<i>Birthplace</i>	<i>Rate per 100,000</i>	
	<i>Male</i>	<i>Female</i>
United Kingdom	27	27
Ireland	97	213
British Caribbean	197	170

JAPAN

The statistics for sarcoidosis in Japan underlines the adage that you will find it if you look hard enough. A Japan Sarcoidosis Committee was set up and carried out five sarcoidosis surveys across the nation from 1960 to 1972 (6). These surveys detected 3215 cases with a rate of 3 per 100,000 population. A sixth survey was completed in 1977, revealing a fourfold increase of the prevalence rate to 12 per 100,000 population (7). The determination, pertinacity and efficiency of this Committee will undoubtedly increase these rates still further in the future.

Based on their figures in various parts of Japan, they feel that sarcoidosis is a disease induced by an infective agent, prevalent in cool climates and influenced by seasonal and ecological variations, and developing in predisposed individuals.

ICEBERG SYNDROME

Sarcoidosis is an iceberg syndrome for many forms of the disease lie undetected (Fig. 1). We must dig deeper to uncover latent forms of the disease. As new techniques emerge, they help to detect excitingly new clinical manifestations of the disease. When fluorescein angiography was introduced, it revealed

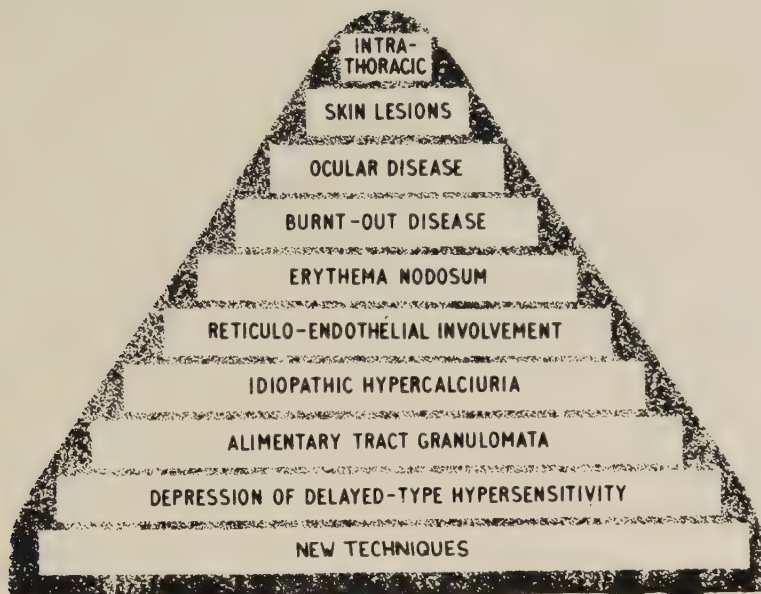


FIG. 1. Iceberg.

leaking retinal veins which were promptly sealed by corticosteroid therapy. Likewise, brain-scans brought a new dimension visualising space-occupying sarcoid granulomas and ventricular block due to them.

Sarcoidosis of the heart is another example for it is difficult to recognise clinically and may only be revealed for the first time at autopsy. It should be suspected if a patient with a multisystem disorder develops heart block, bundle branch block, arrhythmias, congestive cardiac failure, pericarditis or cardiomyopathy. No portion of the heart is immune to infiltration by sarcoid granulomas but the myocardium is by far the most frequently involved, particularly the ventricular free wall, followed by the ventricular septum, the right ventricle, and, lastly, the atrial wall. Involvement of the pericardium and endocardium should be regarded as but an extension of the myocardial granulomatous process.

ETIOLOGY AND PREDISPOSING FACTORS

The cause of sarcoidosis remains unknown. We do not know whether it is one disease or whether there are many contributing factors (Fig. 2).

Infection. Many organisms provoke a non-specific granulomatous reaction but this should not be misconstrued as multisystem sarcoidosis. Helminths

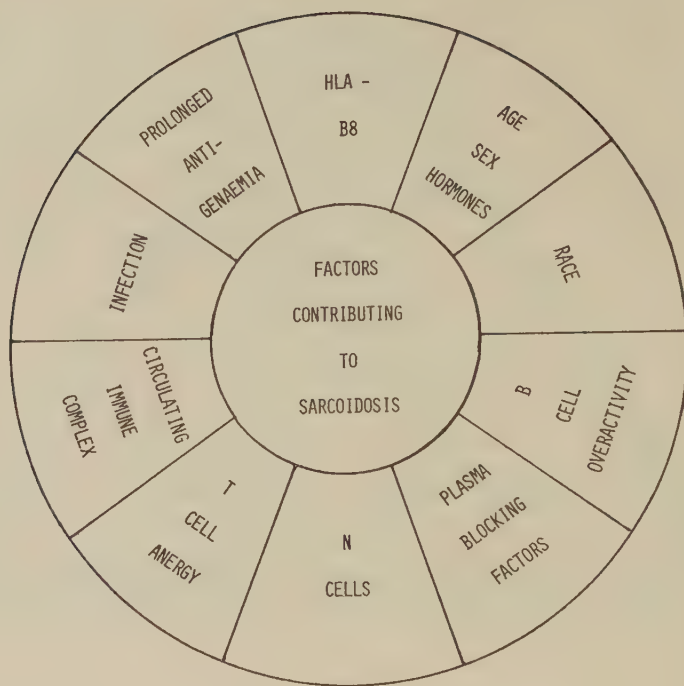


FIG. 2. Etiology.

provoke such reactions in the liver and CNS, anonymous mycobacteria cause swimming-pool and fish-tank granulomas and *Mycobacterium leprae* produces confusingly similar granulomas. Sarcoidosis was once thought to be caused by the human tubercle bacillus but there are many important points differentiating sarcoidosis from tuberculosis. Fungi and protozoa have also been incriminated.

Claims for a causal virus are longstanding, and at various times, mumps, influenza, para-influenza, Newcastle agent and measles virus particles have been isolated. High titres of antibodies to several viruses have been noted, but this is more likely to reflect lymphoproliferation by exuberant B cells than a viral etiology of the disease.

Genetic and racial factors. The occasional occurrence of familial sarcoidosis suggests possible genetic influences. The evidence suggests a racial predisposition to familial sarcoidosis and recessive mode of inheritance for susceptibility. Our HLA antigen studies also indicate that there is an inherited susceptibility to arthritis and erythema nodosum with B8 as a genetic marker.

Allergy. Inhalation of pine pollen and peanut dust, clay-eating and chewing pine pitch have all been incriminated as contributory regional factors in different areas.

Chemical. Beryllium and zirconium are known to produce sarcoid granulomas in the sensitised individual, but other elements do not seem to have this effect. Exhaustive skin testing with metals and other inorganic elements in sarcoidosis patients and controls has not revealed any peculiar hypersensitivity to chemicals. Skin tests for sarcoidosis, beryllium and zirconium disease and leprosy are very similar in that a sarcoid granuloma is found at the injection site one month after inoculation. Each skin test is individually specific for its own disorder and there is no overlap.

Autoimmune disorder. There is one pattern of sarcoidosis that could conceivably fit, namely erythema nodosum with hilar adenopathy and polyarthralgia, a syndrome associated with a circulating immune complex.

EPIDEMIOLOGICAL SEARCH

These are recommendations for a concentrated search for sarcoidosis and its possible causes in nature:

a) In setting up a sarcoidosis clinic, three disciplines should combine to make it a joint clinic comprising chest physician, dermatologist and ophthalmologist. The increased yield would be fruitful.

b) Mass chest X-ray surveys should be in the wake of tuberculosis and sarcoidosis. Those responsible for the eradication of these disorders should send follow-up teams for the detection of sarcoidosis. It would be of particular interest to know its incidence in the Caribbean and in South America.

c) In addition to chest X-rays, there are other interesting markers of granulomatous inflammation including serum angiotensin-converting enzyme and serum lysozyme levels.

d) Skin test surveys using tuberculin and other recall antigens, and Kveim antigen studies as are being undertaken at present.

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The relationship between hepatitis B surface antigen and primary liver cell carcinoma in the Nigerian African

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SUMMARY

Primary Liver Cell Carcinoma (PLC) is very common in the tropics. The exact aetiology of this tumour is still unknown but compelling epidemiological data indicate a strong association between Hepatitis B surface antigen (HB^sAg) and PLC. A previous report from Nigeria had failed to support this observation. The present study documents a positive relationship between the frequency of HB^sAg and PLC—a pattern similar to that in other countries of high incidence of PLC. Contrary to some reports, there was no relationship between age, sex, HB^sAg and alphafetoproteinaemia (AFP). The implications of these findings are discussed.

Primary liver cell carcinoma (PLC) is a common tumour in Subsaharan Africa and South East Asia (1, 2, 3). It is rare in the Western countries, and in some countries in Central and South America. PLC accounts for 18 per cent of all cancers in the male in Ibadan (4) and for 20% in Ghana (5).

Despite the well recognised variation in geographical distribution and differences in the clinical course of patients with PLC, the exact aetiology of this tumour is unknown. While racial and genetic factors do not appear to play a significant role in the course, there is a compelling but inconclusive epidemiological evidence that several environmental factors may act synergistically to encourage the high incidence of PLC in certain areas of the world. Of the various factors, the most important appear to include aflatoxins, hepatitis B virus infection (HBV) and Hepatic cirrhosis (6, 7).

The association of the post necrotic (macronodular) cirrhosis (PNC) and PLC, is well established. The relationship of these two entities is even more striking in tropical Africa where for instance in Nigeria 62.5% of PLC patients have associated PNC (4).

Although it is generally accepted that viral hepatitis, especially that due to Hepatitis B, is a very important antecedent lesion of PNC, the existence of aetio-

logic association between HBV infection and PLC with or without the intermediate lesion of PNC, still requires a direct evidence.

Evidence of HBV infection, immediate or remote, is usually documented by the detection of certain markers of HBV which include Hepatitis B surface antigen (HB^sAg), Hepatitis B antibodies (HBAb) Hepatitis B Core antigen (HBcAg) antibodies to HBcAg (anti-HBc) e-antigen (eAg) and antibodies to e-antigen (anti-HBe Ag). The methods of detection of these various markers vary in sophistication, sensitivity and reliability. In most published data from the tropics, the detection of HB^sAg has been used as the marker for HBV infection.

Contrary to reports from many countries especially those with high incidence of PLC, an early study from this hospital revealed that the prevalence of HB^sAg in the sera of Nigerian PLC patients was comparatively low and even lower than that found in the healthy blood donor population (9). A further examination of the relationship between HBV infection and PLC is the subject of this communication.

MATERIALS AND METHODS

The subjects of this study comprise 68 patients (59 males and 9 females) seen and treated in the Liver Unit of the University College Hospital (UCH) Ibadan, between October 1976 and January 1978 in whom PLC had been histologically confirmed.

Sera of these patients were tested for the presence of HB^sAg by counter immunoelectrophoresis. Alpha-feto proteinaemia (AFP) was detected according to the method of Smith *et al.* (1971) (10) by double diffusion in agar gel (micro-Ouchterlony's) technique using monospecific antiserum prepared in rabbits. Ten randomly selected specimen of sera from these patients were tested for HB^sAg using the radioimmunoassay method (Austria Abbot Laboratories) (13).

Controls comprised of 2045 healthy blood donors in whom the frequency of HB^sAg had been determined and previously reported (14).

RESULTS

Frequencies of Hepatitis B antigen and Alpha feto-protein

The frequencies of HBsAg and AFP are summarised in Table 1. Fifteen (22 percent) of the patients with PLC had positive HBsAg while 30 (44 percent) had positive AFP).

Sex, Age and Hepatitis B antigen

Thirteen of 46 males (28%) and 2 of 9 females (22%) had HBsAg in their sera. The difference between both sexes is not significant. In the age group

TABLE 1

HBsAg and AFP in 68 patients with PLC

<i>Age in years</i>	<i>No. of patients</i>	<i>HBsAg Positive</i>	<i>AFP Positive</i>
> 40	40	7 (17.5)	17 (42.5)
> 40	40	7 (17.5)	17 (42.5)
All ages	68	15 (22.1)	30 (44.1)

Percentage in brackets

analysis (Table 3) 8 of 28 patients below the ages of 40 years and 7 of 40 patients above 15 age of 40 years had Hepatitis surface antigenaemia. The difference was not statistically significant. The pattern did not alter even when the groups were separated either at 40 (Table 2) or at 50 years of age (Table 4).

TABLE 2

Age, HBsAg and AFP in male Patients with PLC

<i>Age in years</i>	<i>HBsAg Positive</i>	<i>HBsAg Negative</i>	<i>AFP Positive</i>	<i>AFP Negative</i>
40 (23)	8	15	12	11
40 (36)	5	31	14	22
Total (59)	13	46	26	33

TABLE 3

Relationship between Age, HBsAg and AFP

<i>Age in years</i>	<i>HBsAg positive</i>	<i>HBsAg Negative</i>	<i>AFP Positive</i>	<i>AFP Negative</i>
< 40 (28)	8	20	13	15
> 40 (40)	7	33	17	23
Total (68)	15	53	30	38

For HBsAg $\chi^2 = 0.62$ $P > 0.05$ (NS)For AFP χ^2 For AFP χ^2 For AFP $\chi^2 = 0.1$ $P > 0.05$ (NS)

TABLE 4

Age and HBsAg in 68 patients with PLC

<i>Age in Years</i>	<i>HBsAg + ve</i>	<i>HBsAg — ve</i>	<i>AFP = ve</i>	<i>AFP — ve</i>	<i>Total (68)</i>
< 50	13	36	23	26	49
> 50	2	17	7	12	19

For HBsAg $X^2 = 1.22$ $P > 0.05$ (NS)For AFP $X^2 = 0.23$ $P > 0.05$ (NS)*Sex, Age and Alpha Feto-proteinaemia*

Twenty six of 59 males (44%) and 4 of 9 females (44%) had alpha-feto-proteinaemia. There was no statistical difference between the frequency of AFP in patients below the age of 40 years and those above, or those below and those above 50 years of age (Tables 2, 3, 4).

Relationship between HB Ag and AFP

The association between HBsAg and AFP is summarised in Table 5 of 30 patients with positive AFP, 10 (33%) had positive HBsAg while 5 of 38 patients who were negative for AFP had HBsAg in their sera. The difference however, did not achieve statistical significance.

TABLE 5

Relationship between HBsAg and AFP in 68 patients

<i>AFP</i>	<i>HBsAg + ve</i>	<i>HBsAg — ve</i>
AFP + ve (30)	10	20
AFP — ve (38)	5	33
Total (68)	15	53

 $X^2c = 3.88$ $P > 0.05$ (NS)*Radio-immunoassay detection of HBsAg*

Four of 10 patients tested for HBsAg using the RIA method had HBsAg in their blood. Two of these 4 had previously been shown to be positive by the counter immunoelectrophoresis method.

DISCUSSION

In a previous study of 2045 healthy blood donors in our hospital, the prevalence rates of Hepatitis B antigenaemia was determined as 5.1% (12). The frequency of HBsAg in 22 per cent of Nigerian patients with PLC indicate a positive association between HBsAg and PLC. This is at variance with the previous report from this hospital in which the frequency was 4% (13). Whether this represents an increase in frequency of HBV infection in Nigeria is uncertain. Our findings are in agreement with the experience of others in various parts of Africa (14-17), India (10), Greece (19), Taiwan (20) but conflict with reports from Hongkong (21), Singapore (22) and Rhodesia (23) (Table 6).

TABLE 6
Hepatitis B surface antigen and primary liver cancer

Countries	Authors/Year/Ref. No.	Method	PLC Patients		Controls	
			No. Tested	Percentage Positive HBsAg	No.	Percentage Positive
U.S.A.	Smith <i>et al.</i> 1969 (22)	MO	12	0	2412	0.1
Hongkong	Smith <i>et al.</i> 1969 (22)	MO	42	4.7	50	4.0
Greece	Hodziyannis <i>et al.</i> 1970 (20)	MO	13	30.8	—	—
Spain	Terres <i>et al.</i> 1971 (36)	MO	26	34.0	900	0.3
Nigeria	Smith <i>et al.</i> 1972 (14)	MO	50	4.0	29911	6.0
Singapore	Simmons <i>et al.</i> 1972 (23)	ECD	156	1.9	1156	4.1
Ghana	Foli <i>et al.</i> 1977 (18)	COE	114	29.0	980	7.6
Rhodesia	Thomas <i>et al.</i> 1977 (00)	COE	28	0	—	—
Nigeria	Present Study	CIEOP	68	22.1	2045	5.1
U.S.A.	Prince <i>et al.</i> 1970 (15)	IEOP	55	3.6	55956	0.1
Senegal	Prince <i>et al.</i> 1970 (15)	IEOP	218	42.0	1160	9.6
India	Anend 1971 (19)	IEOP	11	63.6	—	0.1
Uganda	Vogel <i>et al.</i> 1972 (29)	CFT	90	40.0	224	30.0
Senegal	Saimot G. 1975 (27)	CEP	117	48.0	99	10.0
Vietnam	Welsh <i>et al.</i> 1972 (30)	IEOP	26	0	—	—
Mali	Maupas <i>et al.</i> 1975 (16)	RIA	21	47.6	40	5

Key: MO = Micro-ouchterlony CFT = Compliment fixation
EOD = Electroosmodiffusion IAHA = Immune adherence haemagglutination
COE = Cross Over Electrophoresis RIA = Radioimmunoassay
IEOP = Immunoelectrosmophoresis

Our preliminary results when the RIA method of HBsAg detection was used support the well established observation that this method is several times more sensitive than the immunodiffusion method (22, 24). It indicates that the various reported rates determined by the latter method are probably underestimation

of the true frequencies of HBsAg in PLC. In nearly all reports where sensitive techniques for the detection of HBsAg have been employed, the frequency of antigenaemia in PLC has been significantly higher than in control groups (25). Furthermore, in studies where other markers of HBV infection such as anti HBsAg, HBcAg, anti HBcAg have been included, nearly all patients with PLC have evidence indicative of past or present HBV infection (15, 26).

Although the number of females in our series was relatively small, our findings support the observations of others that HBsAg in PLC is not related to sex (17, 27, 28). Even though younger PLC patients tended to have a higher frequency of antigenaemia than the older patients the difference was not statistically significant in the present study. This is in agreement with observations from Singapore (22) and Ghana (17) but is in conflict with reports from Uganda where an age dependency of HBsAg was observed (27, 28).

These apparent geographic differences require some explanations. It is possible that this is related to differences in age distributions of PLC patients in different series and populations as previously suggested by Simmons *et al.* (22). The differences may also be quantitative. Relatively insensitive techniques would only detect HBsAg in sera containing high titres while the use of more sophisticated methods would detect lower titres of antigen. This variability in the level of HBsAg titres in PLC patients would support the hypothesis that complexing of antigen to circulating antibody may occur even to the extent that only antibody to HBsAg is detectable in the serum (25).

In our series, AFP was positive in 44% of patients with PLC. This is similar to the experience of 46.5% in Rhodesia (23) and of 42% in Vietnam (29).

Reporting correlation between HBsAg and AFP, Vogel *et al.* (27, 28) emphasised the relevance of analysis of ABsAg in AFP positive and AFP negative individuals. We found no evidence in the present study to support this relationship, or emphasis. Similarly, Nishioka *et al.* (24), Massayeff *et al.* (30) and Foli *et al.* (17) and Thomas *et al.* (23) had shown that the frequency of AFP is not related to the frequency of HBsAg. Contrary to reports of others (31, 32) including those from Uganda (27, 28) and Ghana (17) the present study shows no relationship between age and AFP or between AFP and sex.

Viewed globally, a significant correlation has emerged between the prevalence of PLC and endemicity of HBV as determined by the carrier state of HBsAg. It has been suggested that HBV infection leads to chronic liver damage in the presence of susceptibility factors such as age of onset of infection, duration of infection, immunologic determinants, malnutrition, virulence of organism and chronic exposure to hepatic carcinogens (33).

Despite the compelling epidemiologic evidence, the above hypothesis requires unambiguous data establishing the progression of Hepatitis B infection to PLC.

Such data must show that the infection precedes the development of cancer, that the tumour cells contain virus particles or antigen, and that the virus can transform cells in cultures or induce hepatocellular carcinoma in experimental animals.

In the light of the present observations, however, areas of high incidence of PLC must make an attempt to reduce endemicity of HBV infection in the population. A noticeable reduction in the prevalence of PLC in such areas will serve as a further epidemiologic though indirect proof of cause and effect relationship between HBV and PLC.

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SATELLITE SYMPOSIUM
NEW ACQUISITIONS ON VITAMINS
DURING TREATMENT

Therapeutical actions of the vitamins

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During the last years the study of the therapeutical actions of the vitamins has drawn most of the attention of the vitaminologists all over the world; nowadays, the utilization of the vitamins in therapy and actually as drugs is so well established that in a large number of diseases the vitamins are chosen as therapeutical agents; vitamin B12, vitamin E, pantothenic acid, pyridoxine, and nicotinic acid play, when given at appropriate doses, a remarkable role in therapy; in these cases advantage is taken of the characteristics of the vitamins bound to their pharmacological action.

The controlled use of the vitamins as drugs must be looked upon in a very favourable way since it involves the therapeutical action of compounds which although they are produced by synthesis, can be identified with certain constituents of the living matter. The molecules of the vitamins are subject to an easy and more or less rapid metabolism: in the body they follow a natural path which leads them to be absorbed, transported by the biological fluids, transformed within the cells, and finally eliminated by physiological mechanisms only.

The effects and the actions determined by the vitamins as drugs are basically due to exaltation and potentiation of physiological functions or to the physiological stimulation of organs and groups of organs.

Moreover, the vitamins are active at relatively low levels of intake and, being natural compounds, their toxicity is minimal.

The therapeutical treatment with vitamins has virtually no side-effects, even when it is carried on for a long time; furthermore, vitamins don't give addiction.

The effects of ascorbate on normal and abnormal neutrophil motility *in vitro* and *in vivo*

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Stimulation of neutrophil motility *in vitro* has been reported by Goetzl *et al.* (1974) and by Sandler, Gallin and Vaughan (1975). Increased neutrophil motility following ingestion of ascorbate in one child with Chediak-Higashi syndrome and two adult controls has been reported by Boxer *et al.* (1976). However overall there is a paucity of data on the precise effects of ascorbate on neutrophil motility *in vitro* and *in vivo*.

The present study was undertaken to investigate:

a) The effects of ascorbic acid, calcium ascorbate and sodium ascorbate at a concentration range of 10^{-6}M - 10^{-1}M on normal neutrophil motility, using the Boyden technique for the *in vitro* evaluation of chemotaxis.

b) The effects of concentrations of calcium ascorbate and sodium ascorbate which stimulate normal neutrophil movement on the defective motility of neutrophils from 10 patients with recurrent bacterial and viral infection *in vitro*.

c) The effects of ingestion of increasing weekly doses of ascorbate (1 g daily/1 week, 2 g daily/1 week and 3 g daily/1 week) on neutrophil motility in five adult volunteers.

d) Four children and two adults with recurrent infection associated with impaired neutrophil movement were treated with high dose vitamin C (1 g daily for the children and 3 g daily for the adults) for the 3-6 months. Neutrophil chemotaxis was assessed on at least two occasions pre-ascorbate and at one monthly intervals thereafter.

e) Two related kindred with chronic granulomatous disease, severely impaired neutrophil chemotaxis and hyperimmuno-globulinaemia E were treated with vitamin C (1 g daily for six months) and tests of neutrophil function and serum IgE levels repeated at one monthly intervals.

In vitro studies showed that ascorbate and calcium and sodium ascorbate caused considerable enhancement of neutrophil motility. However a fairly high concentration of ascorbate was required to stimulate locomotion. With ascorbate

and sodium ascorbate optimal stimulation was observed at a concentration of $10^{-1}M$ and with calcium ascorbate at $5 \times 10^{-2}M$. The stimulation was dependent upon the presence of serum in the cell suspending medium. The nature of the serum factor has not yet been identified, but is probably not albumin.

When the neutrophils of 10 patients with recurrent bacterial infection and impaired locomotion were treated with calcium ascorbate ($5 \times 10^{-2}M$) and

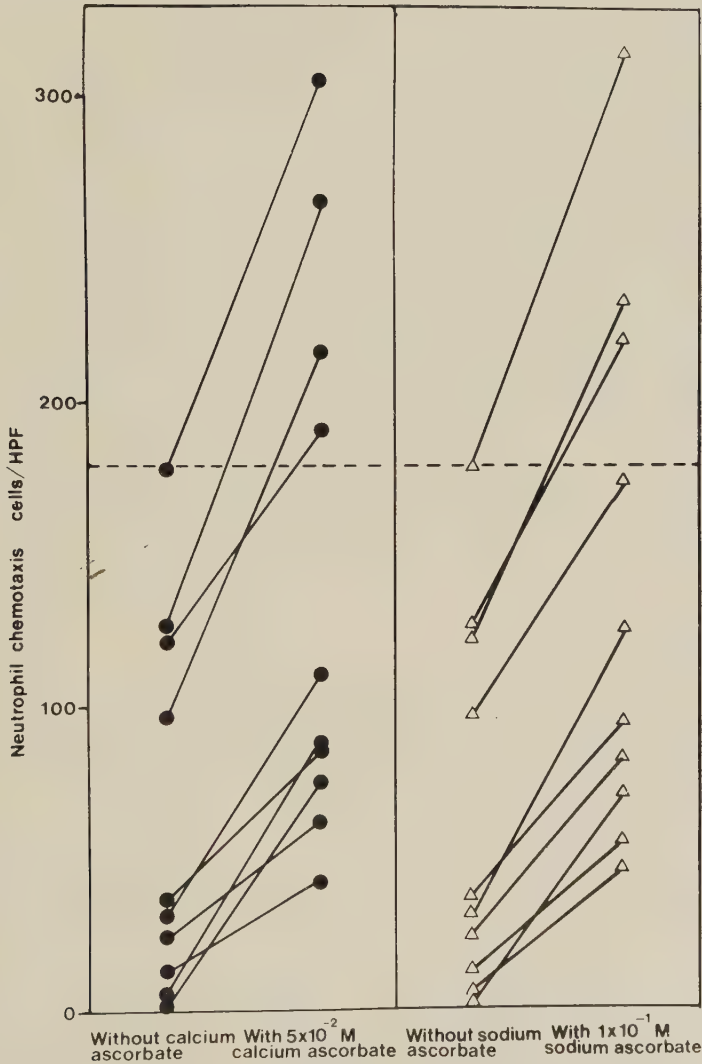


FIG. 1. The effects of calcium ascorbate and sodium ascorbate on the chemotactic responsiveness of neutrophils from 10 patients with defective motility *in vitro*.

sodium ascorbate ($10^{-1}M$) in the presence of 5% fresh autologous serum *in vitro* considerable stimulation of motility was observed (Fig. 1).

These results indicate that ingestion of fairly large doses of ascorbate *in vitro* may be necessary for stimulation of motility *in vivo*. Indeed studies on normal adult volunteers demonstrated a requirement for the ingestion of 2 g-3 g daily of ascorbate. Following ingestion of these doses of the vitamin considerable and consistent stimulation of motility was observed. *In vivo* correction of defective neutrophil motility in six patients with recurrent infection was also observed following ingestion of ascorbate, over a 3-6 month period. The improved cell motility correlated with clinical improvement in 4/6 patients.

Two children with CGD, raised serum IgE and impaired neutrophil motility received 1 g ascorbate daily. Increased hexose monophosphate shunt activity, neutrophil motility and a progressive decrease in serum IgE levels was observed over a six month period. These children also received prophylactic antibiotics (Bactrim) which were always stopped 1 week prior to assessment of neutrophil function. However *in vitro* testing showed that sulfamethoxazole and trimethoprim either individually or in combination had no inhibitory effect on neutrophil or lymphocyte function *in vitro*. Since ascorbate has been included in their treatment (six months) both children have remained free of infection over a period which included the winter months and have manifested a distinct "growth spurt".

In conclusion ascorbate causes increased motility of normal and defective neutrophils both *in vitro* and *in vivo*. However fairly large doses of the vitamin are required to achieve stimulation.

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Vitamins of the group B and metabolic diseases

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Vitamins of the B group

EXTENT

This topic implies the prior definition of the extent of the group of vitamins envisaged here. In fact, since the discovery by Wildiers of the bios in 1901 and the recognition by Emmel in 1920 that the so called vitamin B was composed of at least two different substances, not less than 15 elements have been identified in this group, of which it may be said that they are all water-soluble substances, that they originate mostly from common natural sources, that their molecules all contain an atom of nitrogen and that they probably all have coenzyme functions or are parts of coenzymes.

Of the 15 numbered elements, 8 belong to the conventional vitamins, accepted by the IUPAC (International Union of Pure and Applied Chemistry), 7 fail

<i>Code</i>	<i>Conventional Vitamins</i>	<i>Vitaminoids (paravitamins)</i>
B ₁	Thiamin	—
B ₂	Riboflavin	—
B ₃ (PP)	Niacinamide	—
B ₄	—	Adenine
B ₅	Pantothenic acid	—
B ₆	Pyridoxine	—
B ₇	—	Choline
B ₈ (H)	Biotin	—
B ₉ (Bc)	Folic acid	—
B ₁₀	—	Paba
B ₁₁	—	Carnitine
B ₁₂	Cobalamin	—
B ₁₃	—	Orotic acid
B ₁₄	—	Xanthopterin
B ₁₅	—	Pangamic acid

FIG. 1. Vitamins and vitaminoids of the B group.

only by one detail to enter the official group, or are considered by some but not all countries as being vitamins, or have been regarded as vitamins in the past and are therefore called vitaminoids or paravitamin factors.

The first slide shows a compilation from vitamin B_1 to the paravitaminic factor B_{15} .

FUNCTIONS

The participation of all the vitamins of the B group in intermediate metabolism, namely in the Krebs cycle, is well known.

Fig. 2 summarizes the main points of activity of the most representative of these vitamins.

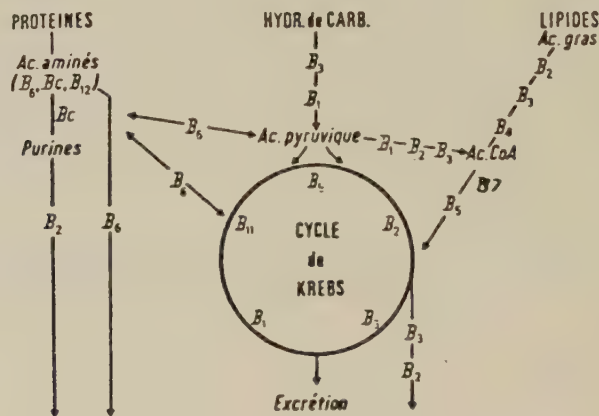


FIG. 2

At the mentioned places, vitamins of the B group have each a definite biochemical mode of action.

This mode of action is summarized in the next slide (Fig. 3).

METHODS

Now, before envisaging any mechanism of interaction between vitamins and metabolic diseases, it is essential to say a word about how the status of vitamins may be investigated and how far conclusions are reliable.

<i>Code</i>	<i>Chem. designation</i>	<i>Mode of action</i>	<i>Active form</i>
B ₁	Thiamin	Decarboxylation of α -ketonic acids	TPP
B ₂	Riboflavin	H ₂ and electron transfer	FMN, FAD
B ₃ (PP)	Niacinamide	H ₂ transfer	NAD, NADP
B ₄	Adenine	Redox	NAD
B ₅	Pantothenic acid	Acyl transfer organic acid-activator	CoA
B ₆	Pyridoxine	transamination, amino-acid decarboxylation	PSP
B ₇	Choline	Transmethylation	acetylcholine
B ₈ (H)	Biotin	CO ₂ transfer	
B ₉ (Bc)	Folic acid	Formic acid and aldehyde transfer	THFA
B ₁₀	PABA		
B ₁₁	Carnitine	Methyl donor?	
B ₁₂	Cobalamin	Methyl radical transfers	coenzyme B ₁₂
B ₁₃	Orotic acid		
B ₁₄	Xanthopterin		
B ₁₅	Pangamic acid		

FIG. 3. Vitamins of the B group.

To those who could think that measuring the level of vitamins in blood would be a simple and reliable method let us recommend they take into consideration that, for instance, the level of vitamin B₁₂ in blood increases after irradiating the liver with X rays. It would however never come to mind that X-irradiating the liver may improve the status of vitamin B₁₂; this is only one striking example among many others.

It is thus clear, that blood levels of vitamins must be accepted with a certain reservation when the circumstances of their measurement have not been taken into consideration.

Moreover the method used for their determination, whether a chemical or a microbiological one, may be subject to interferences.

Also, it should be remembered that the circulating levels of vitamins are not always correlative of the cellular levels, as slide 4 shows (mainly when aged membranes become less permeable).

Certain methods have therefore been developed to assess vitamin concentrations in accessible cells (such as leucocytes or erythrocytes). The standard error may however be fairly high.

Tissue analysis of animals are of course a sound method to assess the vitamin status (of animals) but, how far is the extrapolation to humans reliable?

VITAMIN EVOLUTION IN HUMAN SYSTEM

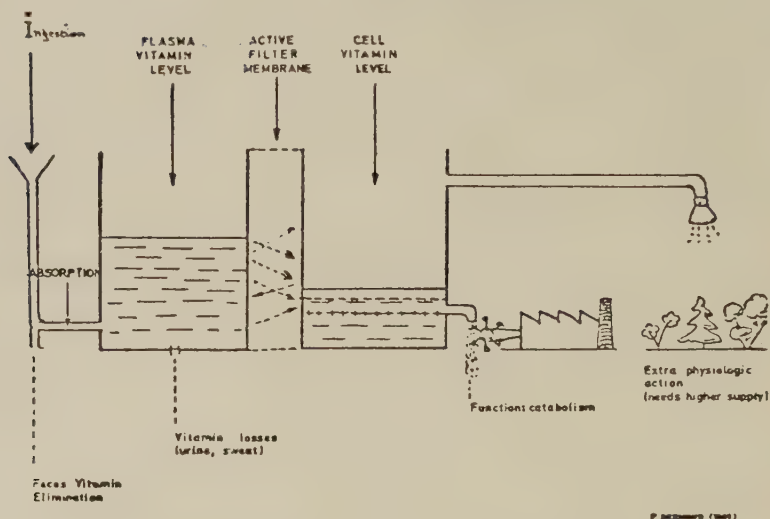


FIG. 4. Diagrammatic transit of the vitamins through the body.

Saturation methods with the assay of the excreted vitamins in urine may have a certain meaning for research purposes. They are less easy to perform as routine analysis. One should also be aware of which metabolites are found in the urine and whether they represent a meaningful proportion of the whole metabolism concerned.

Progress has been realized during recent years by elaborating enzyme function tests.

These tests are based on the coenzymatic participation of vitamins of the B group in holoenzymatic reactions of the biochemical systems of erythrocytes.

The enzymatic activity of a reaction chosen for its specificity is measured *in vitro*, then measured again after the vitamin coenzyme has been added. Finally the quotient of both values is expressed as a coefficient alpha which, theoretically, is a measure of the vitamin status considered.

Such tests are available for vitamin B₁ (21), vitamin B₂ (22), vitamin B₆ (23) and are in development for other vitamins of the B group.

However it should be mentioned that interferences with other metabolisms exist. Slide 5 summarizes the methods available nowadays, slides 6, 7 and 8 show the reactions used for enzymatic tests.

1. LES DOSAGES

<i>Vitamine</i>	<i>Sang total</i>	<i>Plasma ou serum</i>	<i>Urines</i>	<i>Charge au moyen de la vitamine</i>
A		*		*
		Valable pour situations extrêmes. Abaissé dans carences protidiques		Fonction du taux des β -Lipoprotéines
B1	*	*	*	
		Valeur encore discutée	fonction de l'apport en B ₁	
B2	*	*	*	
		Valeur encore discutée	fonction de l'apport et B ₂	
PP	*	*	*	
		Valeur encore discutée	N-Méthylnicotinamide	
B5	*	*	*	
(ac. pan to)		Valeur encore discutée		
B6	*	*	**	
		Valeur encore discutée	dosage acide pyndoxique	
H (Biotine)				
Ac. Foliq.	**	**		
B12	**	**		** (test isotopique de Schilling)
C		*		* (test de saturation de Gounelle et Raoul)
	* (leucocytes)	Fonction de l'apport récent		
D				
E		**		
K				

2. TESTS FONCTIONNELS

<i>Vitamine</i>	<i>Sans charge</i>	<i>Avec charge</i>
A		
B1	** (voir chapitre suivant)	* Test d'Horwitt (glucose + effort → mesure de lactates et de pyruvates)
B2	** (voir chapitre suivant)	
PP		
Ac. Pant.		* Test d'acétylation (Suifam. PAB)
B6	** (voir chapitre suivant)	** Test au tryptophane
Biotine		
Ac. Fol.		* Histidine (FIGLU)
B12	* Dosage urinaire de l'acide méthylmalonique	
C	* (activité phagocytosique des leucocytes)	
D	— Phosphatase alcaline — Phosphates inorganiques	
E	* Test d'émolyse	
K	** Quick	

* Existence d'une méthode
** Méthode généralement admise pour sa spécificité

FIG. 5. Biochemical assessment of vitamin status in man.

A few years ago we performed in France with Lemoine and Codaccioni on 656 subjects (1) what might be called a tripolar survey, where the biochemical assessment was performed concurrently with a nutritional and with a clinical assessment, as represented diagrammatically in the next slide (Fig. 9).

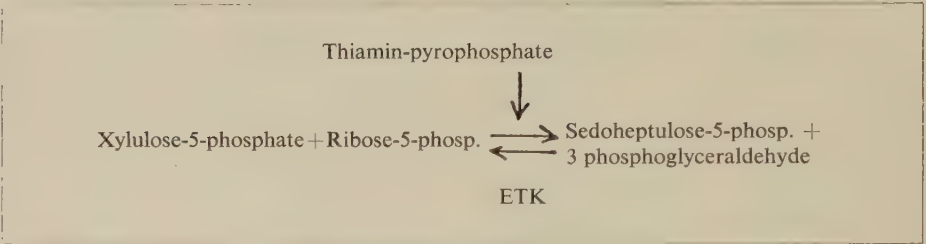


FIG. 6. ETK test.

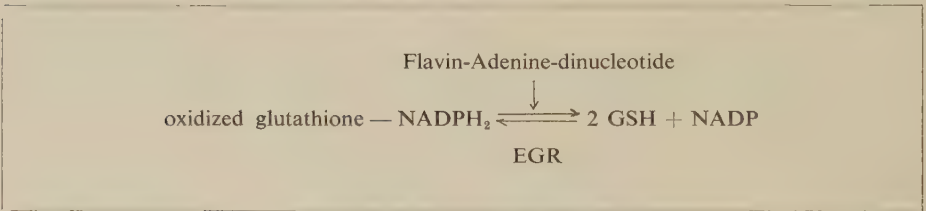


FIG. 7. EGR test.

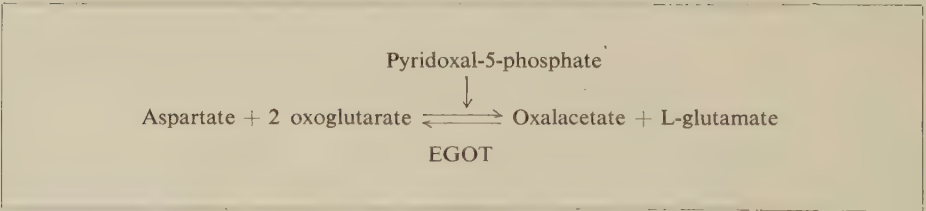


FIG. 8. EGOT test.

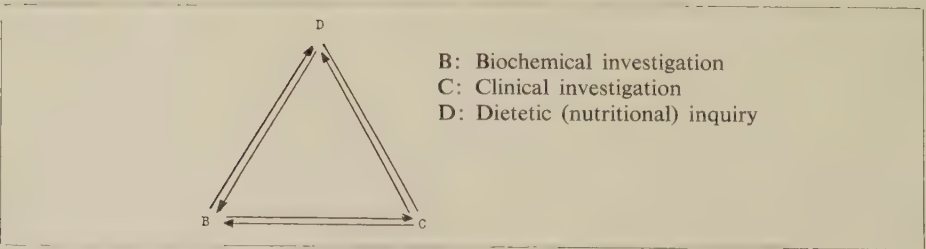


FIG. 9. Diagram of the tripolar test.

As biochemical methods the erythrocyte-transketolase activation test (ETK) has been chosen for thiamin (vitamin B₁), the erythrocyte-glutathione-reductase activation test (EGR) for riboflavin (B₂), the erythrocyte-glutamate-oxalacetate-transaminase (EGOT) for pyridoxine (B₆). Additionally vitamin C has been assayed in plasma.

Despite what we said above, it is interesting to notice here the vitamin C plasma levels correlated significantly with the other investigational methods (nutritional and clinical) at a p level of 0.001.

As regards the tests of the 3 B vitamins checked here, the correlation was also statistically significant for vitamin B₁ and vitamin B₂ (the higher the activation test, the higher the number of clinical symptoms, and the lower the vitamin supply).

As for vitamin B₆, we found a strong interference of alcoholism which paradoxically hyper-normalized the test result. This has been largely confirmed since. This test should therefore be avoided or its interpretation expressed with reservation in the case of alcoholism.

Metabolic diseases

Since we are talking of *alcoholism*, let us consider the *liver diseases* as a first global metabolic disease. The liver is, in fact, one of the main sites for metabolic activity.

In the aforementioned study, where the results of the three main enzymatic tests were compared in controls on the one hand, and on the other hand in 148 patients with liver disease, 106 with disease of the intestinal tract and 235 diabetic of different types, the group with liver disease had the highest frequency of disturbed tests for vitamin B₁, B₂ (and also C), whereas the EGOT test (vitamin B₆), was, for the reasons expressed above, "abnormally normal". Let us notice that pathological biochemical values coincided with alcoholic liver disease, but were not directly correlated with alcohol intake (except for vitamin C).

In a recent publication, Rossouw *et al.* (2) studied 34 patients with *fulminant hepatic failure* and 33 with *decompensated chronic liver disease*, by means of the ETK-activation test for vitamin B₁, plasma pyridoxal-5-phosphate for vitamin B₆, whole blood nicotinamide-adenine-dinucleotide as well as N¹-methyl-nicotinamide urinary excretion for vitamin B₃ (PP).

It is interesting that the authors found an increase in the circulating pyridoxal-5-phosphate in 75% of the patients with fulminant hepatic failure and a decrease in 87% of the patients with decompensated chronic liver disease. The increase in the first case is obviously related to the discharge of hepatic stores of vitamin B₆. Vitamin B₁ was decreased in both types of hepatic failure, however it was more decreased in the decompensated chronic liver disease, whether alco-

holic or not, but the deficiency was higher in the alcoholic group where the average ETK coefficient reached the pathological limit and where the basic enzyme activity was significantly lower than in the control group.

The 235 *diabetic patients* investigated in our tripolar study showed a better vitamin status than the two other pathological groups (as far as vitamin B₁, B₂ and C are concerned).

Within this group, patients with maturity onset diabetes were found to be more often deficient than those with juvenile diabetes; this appears to be in contradiction with results found previously by Haugen (3) who found a worse situation in insulin-dependent diabetes. The role of vitamin B₁ in the metabolism of carbohydrates has long been known. Nevertheless, an uncritical use of its coenzyme, concurrently with an inadapted insulin treatment (insulin remains the basic treatment of keto-acidosis) may have cast an unjustified discredit on this vitamin. One should however continue to consider that, beside the essential insulin and glucose infusions, cocarboxylase (= thiamin pyrophosphate) remains a recommended complement as expressed by Lenti (10).

Standl *et al.* (4) observed that disturbances of the carbohydrate metabolism occurring after administration of hydrochlorothiazide could be improved by oral treatment with vitamin B₁. Lenti and Palano observed a few years ago that raising insulin treatment to an adequate level might normalize a deficient thiamin status in some cases (10).

A series of publications report a decrease of circulating vitamin B₁₂ after chronic administration of biguanides as anti-diabetic treatment (5).

However, Berger (6), investigating 22 patients taking metformin and buformin for 1 to 2 years found no vitamin B₁₂ deficiency with an isotopic dilution method. Conversely, Khan *et al.* (7) and Kanaghinis *et al.* (8) found vitamin B₁₂ deficient levels in diabetics taking no biguanides.

In alterations of carbohydrate metabolism, recent studies have highlighted the role of *pyridoxine* (B₆).

Since the studies of Wynn *et al.* (9), several authors have shown that daily doses of 20 to 100 mg of vitamin B₆ can improve *gestational diabetes* as well as the carbohydrate disturbances provoked by the regular intake of *oral contraceptive agents*. The metabolism of tryptophan is involved here.

It should be stressed that the tryptophan metabolites present in urine in the case of vitamin B₆ deficiency have been proved to be cancerogenic for the bladder.

An increase of the insulin secretion is usually found in the conditions considered above, but some results are in disagreement with this conclusion. As yet no general consensus exists as to whether vitamin B₆ improves diabetes when unrelated to a sexual hormone process. A double blind trial will be completed next year.

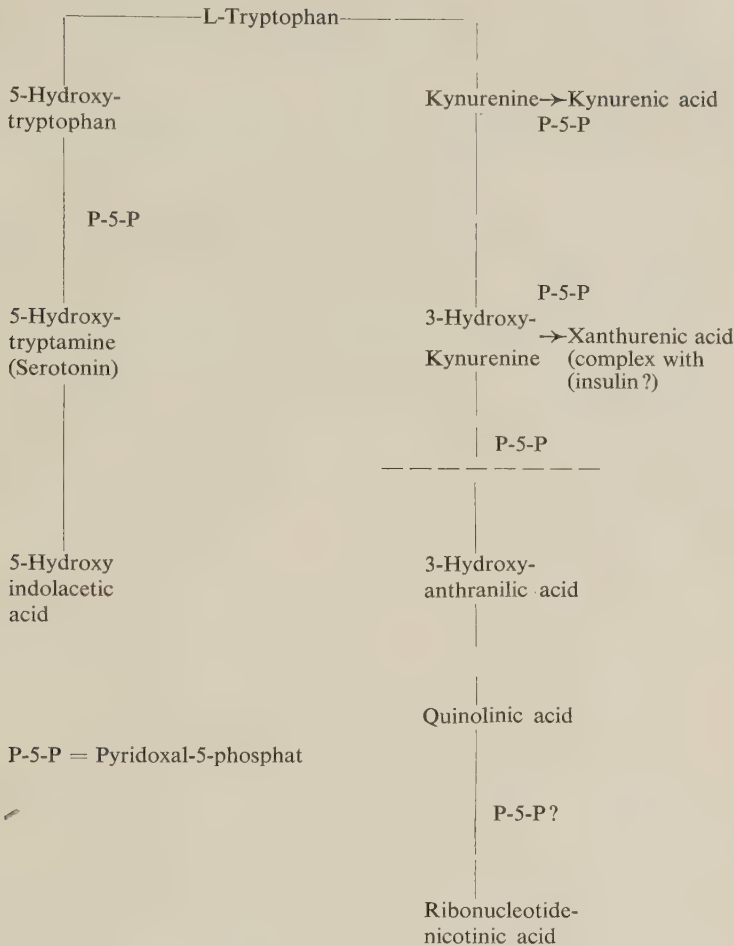


FIG. 10. Diagram of the tryptophan metabolism.

It would not be reasonable to leave the field of diabetes without recalling that, of the three metabolites of *vitamin PP*, alias B_3 , nicotinic acid and nicotinic alcohol have a diabetogenic action, whereas the third one, nicotinamide has no action or a slightly beneficial one. The alteration of the carbohydrate metabolism elicited by the former metabolites seems to be correlated with a reversible suppression of insulin secretion. However if those two vitamin metabolites have a somewhat deleterious effect on the metabolism of sugars, they conversely have a beneficial influence on the metabolism of fats in lowering the blood level of cholesterol and triglycerides. This is generally agreed and needs no further demonstration here.

In the field of *fat metabolism*, former Russian studies seem to have shown beneficial effects of two vitaminoids:

- *orotic acid* (potassium orotate) once known as *vitamin B₁₃* (11) and
- *pangamic acid* (12) known in Russia as *vitamin B₁₅*.

Confirmation of these findings is needed.

Let us go now a step forward to the *metabolism of oxalates*.

Pyridoxine (B₆) catalyzes, as coenzyme, the transformation of glyoxylic acid into glycine rather than oxalic acid.

In B₆-deficient rats, administration of glycine aggravates oxaluria. Gershoff and Prien reported in 1962 that they could reduce oxaluria in man by administration of vitamin B₆. This was confirmed by Mugler in 1970 (13) as visible on the following slide (Fig. 11).

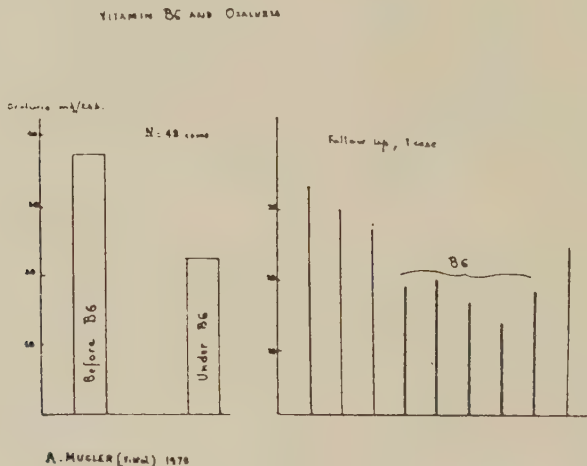


FIG. 11

Nicotinic acid reduces fractional clearance of uric acid and substantially antagonizes uricosuric effects of sulfinpyrazone and of iopanoic acid (15). Folic acid has been tried recently in the treatment of gout but it was concluded that it would not be able to replace allopurinol (16).

Orotic acid (vitaminoid B₁₃) at a dose of 6 mg/day was shown to inhibit purine biosynthesis. Moreover, the administration of allopurinol elicits a substantial orotiduria, thus suggesting the advantage of adding this vitaminoid.

Certain inborn *errors of metabolism*, although moderately frequent, constitute an interesting scientific field for vitamins, which in some cases are the only specific treatment:

— *Vitamin B₁*, does not change the branched-chain amino-acidaemia in *maple-syrup urine disease* but, administered at a dose of 100 mg/day, relieves urinary keto-acidosis within a week (17) [although studies on fibroblast strains show that the defect on the decarboxylase function of branched-chain alpha-keto acid dehydrogenase is located at different functional subunits of the multienzyme complex (18)].

— *Vitamin PP or B₃* has already been mentioned in the treatment of *hyperlipaemia*. This is valid whether hyperlipaemia is a primary or secondary syndrome.

— *Congenital alteration of tryptophan metabolism* results in oligophrenia. This metabolic defect can be controlled by high doses of *vitamin B₆* (19).

— *Cystathionine synthetase deficiency* resulting in homocystinuria may provoke various clinical symptoms such as poor eyesight, mental retardation, seizures, lethargy, thromboses, skeletal abnormalities. In a survey carried out in New South Wales, Wilcken and Turner found 1 case per 58'000 persons but they think that the incidence is higher. Of 27 cases, 8 responded to *vitamin B₆* (20).

This enzyme deficiency may respond in some cases to high doses of *vitamin B₁₂*.

— *Methylmalonic aciduria* has been ascribed to 4 possible pathway defects (24):

1. Methylmalonyl-coA-racemase.
2. Methylmalonyl-coA mutase apoenzyme.
3. Synthesis of desoxyadenosyl-cobalamine.
4. Disturbance of an earlier level of cobalamine metabolism which causes defective synthesis of both *vitamin B₁₂* coenzymes.

Defects 1 and 2 are not vitamin dependent, defect 3 responds to high doses of injected *vitamin B₁₂*. Defect 4 is associated with pronounced homocystinuria. In addition to a dietary reduction of precursor amino-acids, a treatment with *vitamins B₆ and B₁₂* may be tried here.

Lipid storage myopathies have been ascribed to a *carnitine (or B₁₁)*-dependent inborn error of metabolism.

In treating inborn errors of metabolism, vitamin supplementation must be pursued for a certain time before a conclusion can be drawn and, if effective, it will obviously have to be given indefinitely or at least during the whole growth period.

The time is too short to enlarge on the field of the interferences between vitamins and metabolic diseases, but I think the main features have been sketched here.

Summarizing the whole, let us put the stress, beside the traditional indications of vitamins of the B complex in metabolic diseases and in inborn error of metabolism, on the new indications of pyridoxine in alterations of carbohydrate balance.

Finally it should not be forgotten that, additionally, all vitamin deficiencies are also metabolic diseases "per se".

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Therapeutic actions of pantothenic acid, vitamin E and vitamin C

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Research on vitamins carried out in the last few years within the General Physiology Institute of the Faculty of Pharmacy of Rome University has been framed in such a way as to make further contributions to knowledge of the mechanism of action, the physiological and also the therapeutic action of the individual vitamins.

As regards pantothenic acid, we considered the role that this vitamin plays on trophism and structure of hair and on the production of corticosteroid hormones.

Research on vitamin E considered the relationships between blood content of the vitamin and plasma lipids, and research on vitamin C the role the latter carries out on the lipid metabolism and that of cholesterol in particular; furthermore the behaviour of the levels of vitamin C in the plasma and in the leucocytes of adult subjects was followed over different periods of the year.

Within the framework of the research we have been carrying out for some years now on the functions of pantothenic acid (1, 2, 3, 4, 5, 6), we considered it interesting to study any alterations occurring in the hair of rats for want of this vitamin, with the aid of the electronic scan microscope.

In fact it is wellknown that early deficiency signs may be observed mainly in respect of the skin and especially of its annexes, with loss of pigmentation of the hair and alopecia (7, 8, 9, 10, 11, 12).

Every 15 days samples of hair were taken from the backs of the animals fed on a diet wanting in pantothenic acid until the general conditions of the animals in question were extremely affected.

The samples of hair were selected under the optical microscope and then photographed with a Cambridge Stereoscan S-4 electronic scan microscope having an acceleration potential of 20,000 Volts, at enlargements ranging from 200 to 5000 times.

As from 15 days from the beginning of the experiment the keratinic tiles lift up from the stem of the hair, starting to break, as may be noted in Fig. 1. Thereafter the deformations of the keratinic coat become more obvious, with

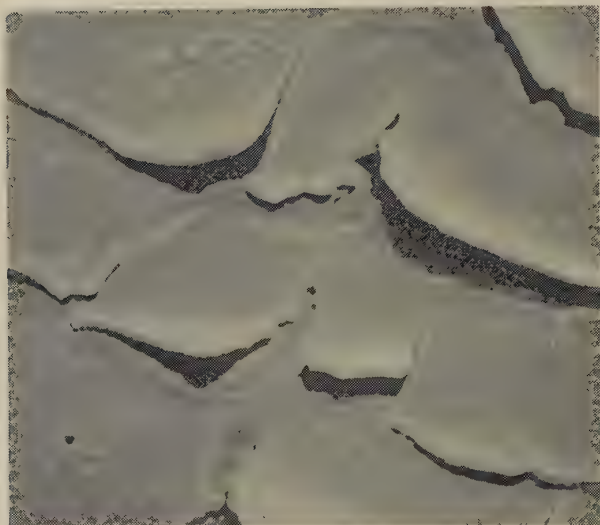


FIG. 1 ($\times 5000$). Metallisation realised with Au.

the appearance of more widespread breaking and flopping of the hair longitudinally; finally after 55 days the whole surface of the hair tiles is involved with a vast number of breakages in all directions (Fig. 2).

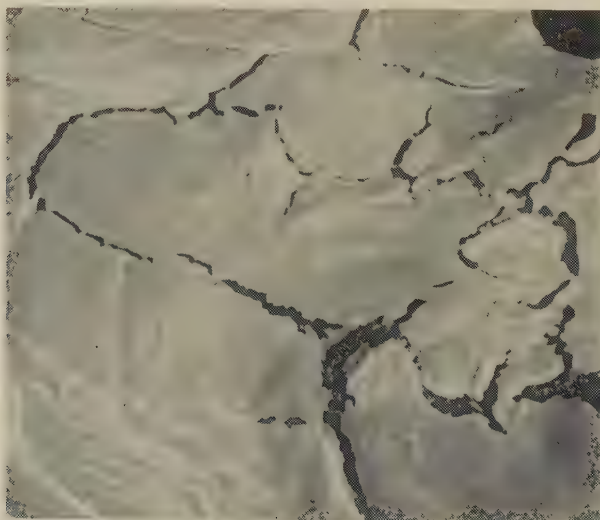


FIG. 2. ($\times 5000$). Metallisation realised with Au.

So that we may conclude that lack of pantothenic acid causes a change to take place in the bodywork of the hair, and that this vitamin is essential for the maintenance of its structure.

In an earlier research (5) we showed that the administration parenterally of a single high dose of sodium pantothenate (1 mg/g of body weight) to male albino rats causes an increase in total corticosteroids in the suprarenal glands up to a maximum 6 hours after administration; accordingly we considered it interesting to further develop this study, determining the individual corticosteroid fractions.

The experiment was carried out on adult male albino rats of Wistar strain: 120 animals were administered intramuscularly a single high dose of sodium pantothenate (1 mg/g of body weight); while the control animals received an injection of physiological solution of the same volume.

One, six, 16 and 24 hours after administration of sodium pantothenate, the animals were sacrificed following anaesthesia with ethylic ether.

To determine the corticosteroids, we used a technique based on the chromatographic fractioning of the lipid extract of the surrenal, obtained with the Folch method (13, 14). Four successive chromatographies showed the various fractions of the corticosteroids. A mixture of pure corticosteroids was used as reference (15).

The quantitative determination of the corticosteroid fractions was realised spectrophotometrically at 510 μ m by means of tetrazol blue reaction according to the Nowaczynsky technique (16).

The corticosteroids determined were desoxycorticosterone, corticosterone, cortisone and cortisol; the results are given in the table hereafter.

	<i>Control</i>	<i>After 1 hour</i>	<i>After 6 hours</i>	<i>After 16 hours</i>	<i>After 24 hours</i>
Cortisol	1.28 \pm 0.04	2.10 \pm 0.01	4.02 \pm 0.10	3.30 \pm 0.08	2.48 \pm 0.04
Cortisone	1.70 \pm 0.02	0.96 \pm 0.01	3.09 \pm 0.10	2.90 \pm 0.04	2.85 \pm 0.03
Corticosterone	4.54 \pm 0.12	8.74 \pm 1.05	12.21 \pm 2.48	6.07 \pm 1.53	5.14 \pm 1.37
Desoxycorticosterone	3.04 \pm 0.11	0.28 \pm 0.04	2.05 \pm 0.04	1.57 \pm 0.02	1.10 \pm 0.03

From the results obtained, it may be noted that the synthesis of cortisol, cortisone and corticosterone is markedly influenced by the administration of a single high dose of sodium pantothenate, and that the maximum increase is found six hours following administration of the vitamin. The concentration of desoxycorticosterone, on the other hand, after a sudden initial fall, does not

follow over the period of time considered the value of the averages of the control animals.

As preliminary research for a study of the role carried out by vitamin E in the lipid metabolism as antioxydant of polyunsaturated fatty acids, we determined the hematic level of α -tocopherol in a sample of the Italian population represented by healthy subjects, all of whom males, of various ages (18-24; 25-54; > 55 years of age).

On the basis of the correlation showed by various authors (17, 18, 19, 20) between the levels of α -tocopherol and those of β -carothene and of the plasma lipids, we also determined the β -carothene, total lipids, total cholesterol, β -lipoproteins and triglycerids.

Determination of total α -tocopherol and of β -carothene was realised with the method proposed by Rindi (21). The levels of α -tocopherol we obtained presented an average value of 0.96 for individuals aged between 18 and 24, a value of 1.14 in the individuals aged 25 to 54, and 1.08 mg/100 ml in subjects over 55 years of age. No value was found under the limit of 0.5 mg/100 ml.

The difference found between the first and the second group proved significant (P 0.01), while as regards the fall encountered between the second and third group cannot be considered so.

Furthermore it is seen that the levels of α -tocopherol in the subjects of the first group mainly fall within the interval 0.80-0.99; while for the age group included between 25 and 54 years of age, a high percentage of subjects (over 50%) show values of over 1.20.

Finally the coefficients of correlation between all the variables examined were calculated; more particularly it may be noted that tocopherol is significantly correlated with age and with cholesterol, in agreement with the findings of other authors (22, 23, 24).

Within the framework of research carried out on the role carried out by vitamin C on the lipid metabolism, we considered the effect on man of the administration of high doses of ascorbic acid above all with reference to the serum levels of cholesterol, total lipids and triglycerids.

In man the normal limit values of the parameters we considered are 250 mg of cholesterol per 100 ml of blood, 1000 mg of total lipids per 100 ml of blood, and finally 172 mg of triglycerids per 100 ml of blood.

Our research was carried out on subjects aged 65 to 90, of male sex, to whom we administered 3000 mg per day of ascorbic acid for three weeks. These subjects prior to treatment showed an average concentration in the plasma of 0.17 mg % of ascorbic acid, and 0.77 γ /mg of proteins in the leucocytes.

The analyses were carried out: in the case of ascorbic acid, with the Albanese colorimetric method (25), in the case of cholesterol, with the Roeschlau method

(26), total lipids with the Zoellner method (27), and triglycerids with the Eggstein method (28).

Following the administration of ascorbic acid, the cholesterol falls in a first group from 225 to 194 mg %, and in a second group from 290 to 249 mg %; total lipids from 891 to 788 mg % and from 1110 to 860 mg %; and triglycerids from 141 to 111 mg % and from 241 to 179 mg %. These falls are statistically significant in all the subjects considered.

On the other hand the concentration of ascorbic acid rises significantly in plasma from 0.17 to 0.24 mg %, and to a slighter extent from 0.91 to 1.07 γ /mg of proteins in the leucocytes.

A preliminary investigation on 11 volunteers, aged 20 to 30, consisting of 6 men and 5 women, was carried out for the purpose of ascertaining any variations in the levels of ascorbic acid in the plasma and in the leucocytes over different periods of the year.

The determinations on the content of vitamin C in the plasma and in the leucocytes were carried out at three-month intervals, using the Albanese colorimetric method (25).

Average values of 0.17 mg % of ascorbic acid in the plasma and 1.1 γ /mg proteins in the leucocytes were considered as indices of sufficient saturation.

The results of the determinations made gave the following average values of ascorbic acid: in March 0.178 mg % in the plasma and 1.00 γ /mg of proteins in the leucocytes; in June 0.268 mg % in the plasma and 1.11 γ /mg of proteins in the leucocytes; in September 0.272 mg % in the plasma and 1.13 γ /mg of proteins in the leucocytes.

These results show that the concentration of ascorbic acid in the plasma undergoes variations over the period of time considered by us, being significantly lower in March. Whereas limited variations were found as regards the content of vitamin C in the leucocytes.

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Prophylactic use of folic acid and vitamin E in the anaemia of premature infants

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The therapeutical use of folic acid and vitamin E is very interesting in the prophylaxis and the therapy of anaemia in premature infants.

It is known that infants whose weight at birth is low are particularly prone to anaemia. Many causes are involved. A few, responsible of the so called "precocious anaemia of the premature" depend on physiological phenomena typical of this age.

They are: 1) insufficient erythropoiesis determined by a poor amount of medullary substance in the bones of the premature as compared with the rapid growth of the body; 2) the decreased duration of life of erythrocytes which is 1/3rd below the average seen in adult subjects.

Other causes of anaemia depend on pathogenetic mechanisms typical of this age: among these the most common are haemolysis and haemorrhage.

Folic acid and vitamin E take part with a two-fold mechanism in the prophylaxis and therapy of anaemia. The first promoting erythropoiesis through the synthesis of nucleic acids and the second protecting erythrocytes from haemolysis.

Vitamin C and atherosclerosis

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The problems of overnutrition in the rich industrialized societies often overshadow the fact that diets rich in calories can be deficient in catalytically-active components. In many countries seasonal vitamin C deficiency is probably the commonest form of nutritional disbalance. Permanent chronic marginal ascorbate deficiency is very common in atherosclerosis, diabetes mellitus and in the aged.

The use of the model of a chronic marginal ascorbate deficiency, which simulates human hypovitaminosis C, has shown cholesterol accumulation in the liver and blood plasma to occur in ascorbate-deficient guinea pigs. The investigation of this phenomenon, designed to follow cholesterol distribution between plasma and tissues, absorption of exogenous and synthesis of endogenous cholesterol, excretion of neutral sterols and bile acids, yielded an unequivocal conclusion: vitamin C is necessary for cholesterol transformation into its principal catabolic product, bile acids. In seemingly healthy, but hypovitaminous animals this process becomes slowed down, with the subsequent cholesterol accumulation in the liver, hypercholesterolemia and prolongation of the half-life of plasma cholesterol. In chronic experiments, cholesterol accumulation occurs also in the aorta of hypovitaminous guinea pigs. Ascorbate is required for the rate-limiting reaction of cholesterol transformation to bile acids, microsomal 7 α -hydroxylation of cholesterol. Participation of vitamin C in this reaction is mediated by the ascorbate intervention into the turnover of microsomal cytochrome P-450 in the liver. The rate of cholesterol transformation to bile acids is a function of ascorbate concentration in the liver; the highest rate has been found in animals with a maximal steady-state levels of vitamin C in the liver.

Marginal vitamin C deficiency is capable of inducing hypertriglyceridemia, which augments in chronic experiments and leads to an accumulation of triglycerides in the liver and aorta of hypovitaminous guinea pigs. Vitamin C deficiency is implicated in plasma triglyceride lipolysis and in the triglyceride secretion from the liver into the blood. The half-life of plasma triglycerides labeled *in vivo* with ^3H -glycerol becomes prolonged in hypovitaminous guinea pigs. An enhanced vitamin C intake depresses the concentration of serum triglycerides in various animal species, including primates and man. Similarly as in the case of cholesterol, the most striking hypotriglyceridemic effect is exer-

cised by doses of vitamin C that ensure a maximal steady-state concentration of ascorbate in the tissues.

Vitamin C deficiency interferes with the turnover of two important components of the vascular wall, collagen and glycosaminoglycans. Ascorbic acid is important for the hydroxylation of prolin and lysine during ribosomal synthesis of collagen. A decrease in the amount of collagen was described in the aorta of vitamin C deficient guinea pigs. Chronic vitamin C deficiency produces changes in the glycosaminoglycans composition of guinea pig aorta—a decrease of sulfated glycosaminoglycans, such as chondroitin 4—and 6-sulfate and dermatan sulfate. On the other hand, high doses of vitamin C prevent a decline of sulfated glycosaminoglycans in the aorta of animals fed on atherogenic diet.

HYPOTHETICAL SCHEME OF THE PARTICIPATION OF CHRONIC MARGINAL VITAMIN C DEFICIENCY IN THE DEVELOPMENT OF ATHEROSCLEROTIC PLAQUE

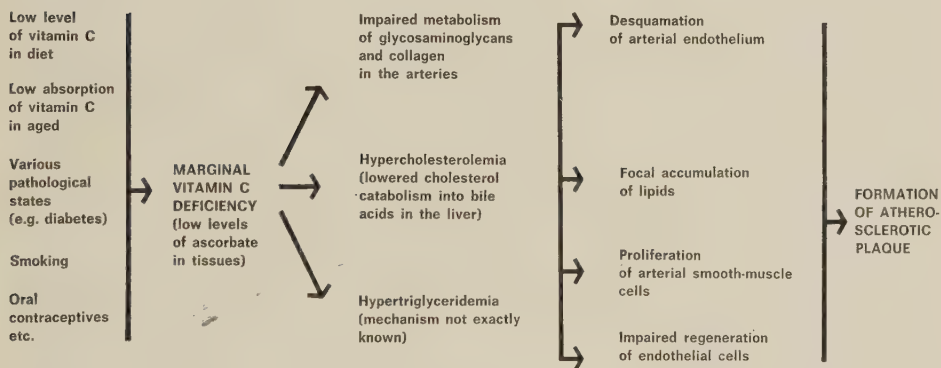


FIG. 1. Hypothetical scheme of the participation of chronic marginal vitamin C deficiency in the development of atherosclerotic plaque.

Changes in glycosaminoglycan and collagen metabolism in vitamin C-deficient arteries may have a profound effect on structural and permeability characteristics of the arterial intima. They can impair the integrity of the connections of the endothelial cells. Separation of endothelial cells was demonstrated electronoptically in the aorta of vitamin C-deficient guinea pigs.

Several authors found pathological changes reminiscent of atheromatous lesions in the arteries of guinea pigs fed only a few weeks on a scorbutogenic diet. In guinea pigs with latent vitamin C deficiency, a cholesterol diet lasting several months led to the formation of advanced atheromas. In experiments of this type large doses of vitamin C did not prevent atherogenesis, but they only retarded this process. Successful prevention of experimental atherosclerosis

with ascorbic acid evidently depends on many factors. An atherogenic diet with a too high cholesterol level is limiting, for example. In guinea pigs fed basal diet without cholesterol addition, which were kept in a state of marginal vitamin C deficiency minimally 3-6 months, edema of the vessel wall, vacuolization of endothelial cells, marked endothelial proliferation, formation of lipophages, focal accumulation of lipids and large intimal musculo-fibrotic plaques were observed in the thoracic aorta and coronary arteries. These results demonstrate that chronic marginal vitamin C deficiency is *per se* capable of producing atheromatous changes in the guinea pig arteries.

Prevention of subclinical hypovitaminosis C opens a prospect of hyperlipidemia being possibly controlled in a large number of people. A hypocholesterolemic action of vitamin C may become manifest in persons with a low vitamin C-status in whom hypercholesterolemia had ensued as a result of a disbalance between cholesterol input into the organism (exogenous and/or endogenous cholesterol), and its elimination in the form of bile acids. The most striking hypocholesterolemic effect was achieved in institutionalized elderly persons and in hypercholesterolemic maturity-onset diabetics after one-year administration of ascorbic acid in doses 500-1000 mg per day. In dietary or pharmacological therapy of hyperlipidemia, an adequate vitamin C supply should be ensured in doses capable of creating maximal tissue steady-state levels of ascorbate. It appears very probable that these doses are several times higher than the officially recommended doses of vitamin C. Definite answer to the question of a possible prevention of human atherosclerosis through optimal vitamin C intake may be given solely after a long-term study on large population groups. There are reasons to include chronic marginal vitamin C deficiency among the risk factors of human atherosclerosis-scheme.

The influence of vitamin C on lipid metabolism

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It is probable that the etiology of arteriosclerosis is multifactorial, hyperlipidemia being a recognized causal factor. It is less well known that vitamin C plays a role in lipid metabolism. Excellent reviews by Ginter on the role of vitamin C in cholesterol metabolism and in the development of arteriosclerosis appeared in 1970 and 1975 (2, 3). Ascorbic acid deficiency raises the plasma cholesterol levels and should therefore also be regarded as being one of the causal factors in the development of arteriosclerosis. We have investigated the behaviour of some lipid parameters in man and in animals with different supplies of ascorbic acid (AA).

In guinea pigs chronic latent AA deficiency may be induced with as little as 0.5 mg AA per animal daily, corresponding to about 17 ppm AA, when the animals are kept on an AA free diet for the previous 2 weeks in order to reduce the AA body pool (4). This small supply still avoids the outbreak of franc scurvy, obscuring diagnosis.

In our guinea pig experiments we could show a significant rise in plasma and tissue cholesterol content without exogenous cholesterol load, even when administering 50 ppm AA, a supply three times higher than the above mentioned 17 ppm intake and without the initial 2 weeks of an AA free period (5).

Later on we have doubled the AA intake once more from 50 ppm to 100 ppm, again without the initial AA free period. In this trial we formed four groups. Group I received 100 ppm AA, group II 1250 ppm AA, group III 100 ppm AA plus 0.16% cholesterol, and group IV 1250 ppm AA plus 0.16% cholesterol in the diet. The experiments were terminated after 23 weeks. The determination of plasma and tissue parameters were carried out according to published routine methods. The following influences of the 100 ppm AA intake in groups I and III were found (Table 1). Compared to the control groups on 1250 ppm AA, mean cholesterol levels in 100 ml plasma rose about 30% in the cholesterol non-loaded groups, about 20% ($p \leq 0.005$) in the cholesterol loaded groups. The triglycerides rose about 70% in the groups without a cholesterol load and about 40% in the groups with exogenous cholesterol load ($p \leq 0.01$).

TABLE 1

Total cholesterol (chol), free chol, triglycerides, free fatty acids (FFA), cholesterol acyltransferase (LCAT) activity and phosphatides (phos) in plasma (mean value \pm SEM): their relation to dietary AA supply and chol load. 16 guinea pigs per group, 23 weeks on diet

Groups	Total chol (mg/100 ml)	Free chol (mM/l)	Triglycerides (mg/100 ml)	Phos (mg/100 ml)	LCAT (chol esterified % h)	FFA (μ g equiv./l)
I. 100 ppm AA	89 \pm 6	0.53 \pm 0.04	92 \pm 16	3.2 \pm 0.2	6.2 \pm 0.4	665 \pm 42
II. 1250 ppm AA	67 \pm 4	0.36 \pm 0.02	54 \pm 3	2.5 \pm 0.1	7.6 \pm 0.5	565 \pm 48
III. 100 ppm AA (+0.16% chol)	135 \pm 7	0.96 \pm 0.06	55 \pm 4	3.7 \pm 0.2	2.9 \pm 0.2	680 \pm 48
IV. 1250 ppm AA (+0.16% chol)	111 \pm 6	0.71 \pm 0.03	39 \pm 5	3.0 \pm 0.2	3.3 \pm 0.3	651 \pm 36

The phosphatides showed a significant rise in those animals with the lower AA intake in the cholesterol non-loaded group as well as in the loaded one ($p \leq 0.005$). Ascorbic acid influenced the lecithin cholesterol acyltransferase activity, which dropped significantly in both groups with the lower AA supply and in consequence free cholesterol in plasma rose in group I by nearly 50% and in group III by 35% ($p \leq 0.005$). This corresponds well to the above demonstrated increase in phosphatides. The free fatty acids were not significantly influenced in the cholesterol loaded groups by AA but tended to decrease in the unloaded groups with the higher AA supplementation.

A significant drop of plasma AA in both 100 ppm AA groups was formed. This result was paralleled in the AA content of the leucocytes (Table 2).

TABLE 2

Ascorbic acid (AA) content of leucocytes and in plasma (mean value \pm SEM): their relation to dietary AA supply and cholesterol (chol) load. 16 guinea pigs per group, 23 weeks on diet

Groups	Leucocyte ascorbic acid (μ g in 10^8 cells)	AA in plasma (mg/100 ml)
I. 100 ppm AA	6.68 \pm 0.6	0.01 \pm 0.01
II. 1250 ppm AA	20.83 \pm 1.4	0.60 \pm 0.05
III. 100 ppm AA (+0.16% chol)	8.18 \pm 1.2	0.02 \pm 0.01
IV. 1250 ppm AA (+0.16% chol)	23.54 \pm 1.5	0.91 \pm 0.27

In the aorta (Table 3) free cholesterol increased by about 30% ($p \leq 0.1$) in group I which was kept on a 100 ppm AA containing diet but not loaded with exogenous cholesterol. In group III with an exogenous cholesterol load, cholesterol concentration increased by 10%.

TABLE 3

Free cholesterol (chol) content of aorta (mean value \pm SEM): its relation to dietary AA supply and chol load. Guinea pig aorta, 5 pooled samples per group, 23 weeks on diet

<i>Groups</i>	<i>Free cholesterol (μg/g aorta)</i>
I. 100 ppm AA	12.44 \pm 2.9
II. 1250 ppm AA	9.66 \pm 2.2
III. 100 ppm AA (+0.16% chol)	13.34 \pm 3.2
IV. 1250 ppm AA (+0.16% chol)	12.02 \pm 3.2

Now how is the situation in man? In the industrialized nations frank scurvy seems to be such a rare disease that even overt cases with extensive haemorrhages are diagnosed very late in the clinic only after measuring AA blood levels as for example in a very recent case of scurvy in a 29-year-old Swiss female, in which we determined the plasma AA level thus verifying the diagnosis (1).

Within the scope of the 3rd Basle study in which besides a medical follow up in an apparently healthy population also several blood parameters—including vitamins—were checked, estimations of AA and cholesterol were done. Table 4 shows the AA plasma levels found. The mean values were 1.00 mg/100 ml in males and 1.18 mg/100 ml plasma in females. But there were clearly deficient AA plasma levels. It is noteworthy that in another study, the so-called Jura study—the Jura is a poorer region of Switzerland—the AA plasma levels in males

TABLE 4

Plasma vitamin C concentrations (mg/100 ml) in the population of the 3rd Basle Study and the Jura Study

	<i>Basle Study</i>		<i>Jura Study</i>	
	<i>males</i>	<i>females</i>	<i>males</i>	<i>females</i>
Mean values	1.003	1.184	0.59	0.93
Standard deviation	0.485	0.395	0.42	0.54
Median values	1.01	1.20	0.45	0.89
Range	0.00-7.60	0.18-230	0.02-3.05	0.08-4.95
Number	837	103	488	475

were about half those found in Basle. In the 3rd Basle study there was a significant negative correlation of AA with free fatty acids, triglycerides, cholesterol and β -lipoproteins (Table 5). In more and less diseased aortas of this population AA and cholesterol concentrations were analysed. In these aortas a significant negative correlation of AA and cholesterol could be demonstrated (Table 6). Moreover, a significant negative correlation of AA and cholesterol was found in all samples (Fig. 1).

TABLE 5

Relationship between plasma vitamin C concentration and lipids. Correlation coefficients 3rd Basle Study

<i>Parameters</i>	<i>N = 487</i>		<i>N = 159</i>	
	<i>Male subjects</i>		<i>Male subjects</i>	
	<i>Receiving no vitamin preparation</i>		<i>Taking vitamin preparations part of the time</i>	
Free fatty acids	— 0.14	$p < 0.01$	n.s.	
Triglycerides	n.s.		— 0.28	$p < 0.001$
Cholesterol	n.s.		— 0.17	$p < 0.01$
β -lipoproteins	0.10	$p < 0.01$	— 0.22	$p < 0.01$

n.s. = not significant

TABLE 6

Cholesterol (chol) and ascorbic acid (AA) content of human aorta (mean value \pm SEM): their relation in more (A) or less (B) diseased human aortas. 10 subjects per group

<i>Diseased aortas</i>	<i>Chol (mg/g aorta)</i>	<i>AA (μg/g aorta)</i>
A (more)	5.08 ± 0.39	10.51 ± 1.55
B (less)	1.26 ± 0.07	19.90 ± 1.60

In an investigation in 10 volunteers, aged between 25 and 40, who took 4,000 mg ascorbic acid daily for 3 weeks a significant depression of the cholesterol levels occurred. It can be seen (Table 7) that a fall in the cholesterol level of about 10% in the lowest limit of the borderline region was effected by 4,000 mg ascorbic acid in healthy persons who by today's standards were adequately supplied with vitamin C. This is demonstrated by a plasma ascorbic acid concentration of 1.03 mg/100 ml. Whereas the cholesterol level fell, the ascorbic acid concentration in the plasma rose.

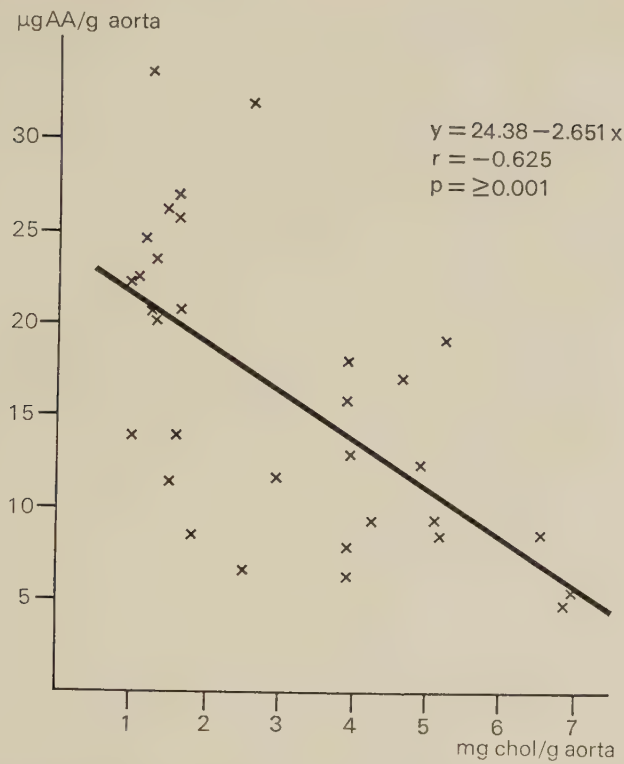


FIG. 1. Correlation of ascorbic acid (AA) and cholesterol (chol) concentrations in human aortas from the Institute of Pathology, University of Basle, 1978.

TABLE 7

Relation of plasma ascorbic acid to plasma cholesterol and blood glucose (mean \pm SEM). 10 fasting volunteers, 25-45 years old. After a period of 1 week without additional AA supply daily intake of 4000 mg for three weeks

Time of assay	Ascorbic acid (mg/100 ml)	Cholesterol (mg/100 ml)	Glucose (mg/100 ml)
Initial	1.03 \pm 0.08	213.3 \pm 10.3	89.9 \pm 2.4
After 3 weeks	1.56 \pm 0.11	194.8 \pm 9.2	85.9 \pm 2.5

In view of the discussion on the etiology of arteriosclerosis, one has to call to mind the opinion, primarily put forward by Yudkin, that carbohydrates, especially glucose, are of causal importance in atherogenesis (8). We therefore investigated the behaviour of the fasting blood levels in relation to intake of ascor-

bic acid. The initial blood glucose levels lie within the normal range. These values are significantly lowered by ascorbic acid, without however reaching hypoglycemic levels (Table 7).

A correlation between ascorbic acid intake and cerebrovascular disease standardized mortality ratios are given in different regions of the United Kingdom (3) in Fig. 2. This clearly illustrates the negative correlation between the mortality ratios and the intake of ascorbic acid.

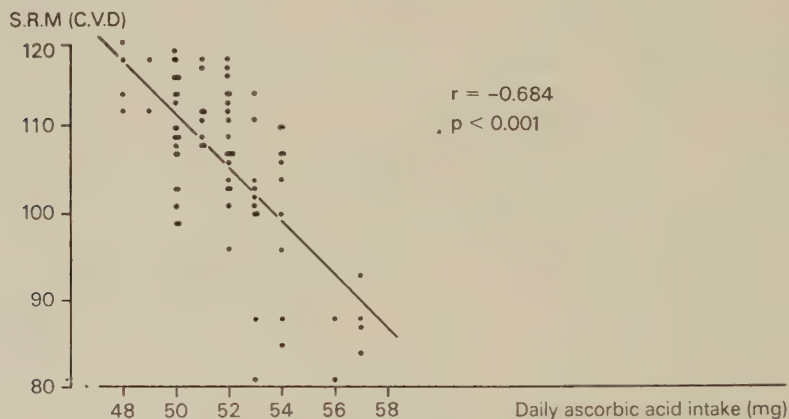


FIG. 2. Correlation between ascorbic acid (AA) intake and the standardized mortality ratios (S.R.M.) for cerebro-vascular disease (C.V.D.) in man (5).

Similar findings were published by Wilson *et al.* (1972), who showed a negative correlation between the vitamin C content of leukocytes and mortality in the aged (9) and by Krumdieck (1974) showing a strong negative correlation between ascorbic acid consumption and cerebrovascular disease and ischemic heart disease (7). Very recently interesting results were reported from Heine and Norden (1978) who treated 63 patients with lipometabolic disturbances out of a group of 202 patients with angiographically proven arteriosclerosis obliterans with 1000 mg AA daily, without a special diet during an average of 16,2 months (3-53 months). This group of patients was divided according to the classification of Fredrickson Hyperlipoproteinemia type IIa and IV were observed most (6).

The whole group showed a significant decrease of the cholesterol from 330 mg/100 ml to 293 mg/100 ml. But the cholesterol serum level only reached normal values when the initial values did not exceed 300 mg/100 ml. In 15 out of 18 patients cholesterol level rose up again to the original level after stopping therapy. A statistically significant decrease in the level of cholesterol and triglyceride was found in type IIa. Also a positive effect was seen in type IIb and IV,

and no effect in type V. The patients treated with AA had the same amount of HDL as the healthy control group. The HDL levels of untreated patients was significantly lower. Clofibrate therapy had no positive effect on the low HDL levels. AA had no side effects, especially no effect on the long-term anti-coagulant therapy with coumarin and indandione, and no increase in creatinine and uric acid levels.

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Lipid peroxidation and cardiotoxicity of adriamycin in experimental animals: a protective role of vitamin E?

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Adriamycin, an anthracycline antibiotic, is one of the most effective anticancer agents currently available and is used in the treatment of several types of cancers (Bender *et al.*, 1978). However, adriamycin chemotherapy is severely limited by the occurrence of cardiotoxicity. Both acute and chronic effects have been described, depending on the dosage and treatment schedule used (Lenaz and Page, 1976). The acute effects are hypotension, tachycardia and arrhythmias, appear to be dose-independent and develop minutes to hours after i.v. administration of the drug. In contrast, the chronic effects develop only after several weeks of treatment with high total dosages of the drugs and lead to the onset of severe, often fatal, heart failure. Different aspects of adriamycin-induced cardiotoxicity have been reproduced in experimental models *in vitro* (isolated rabbit heart) and *in vivo* (mainly acutely treated mice and chronically treated rats and rabbits) (Ghione and Bertazzoli, 1977; Jaenke, 1976, Mettler *et al.*, 1977).

Several biochemical effects, induced by adriamycin mainly *in vitro* have been implicated in the pathogenesis of the tissue toxicity. They include enhanced release of histamin and catecholamines, (which would mainly account for the acute toxic effects); binding of adriamycin to nuclear and mitochondrial DNA, with subsequent inhibition of RNA and protein synthesis; inhibition of Na^+/K^+ -dependent ATP-ase activity; inhibition of reactions utilizing coenzyme Q; alterations in calcium transport and in intracellular electrolyte balance; chelation of divalent cations (Ferrans, 1978).

Recently it was proposed that adriamycin promotes lipid peroxidation by means of reactions mediated by free radical formation (Myers *et al.*, 1977).

In man, rabbits and mice adriamycin-induced cardiomyopathy is characterized by dilatation of mitochondria and myofilaments. This cardiac lesion is strikingly similar to the muscle pathology produced in rabbits and mice by a α -tocopherol (Vitamin E) deficiency, in which the muscular lesions are in part the result of uncontrolled free radical reaction which lead to peroxidation of membrane

lipids (Myers *et al.*, 1976). A similar process of free-radical mediated lipid peroxidation characterises the tissue damage elicited by a number of drug containing quinone functionalities (Handa and Sato, 1975). These drugs are thought to start lipid peroxidation by facilitating the transfer of electrons from endogenous compounds such as NADPH to oxygen, resulting in the formation of hydrogen peroxides. Adriamycin also possess quinone and hydroquinone groups on adjacent aromatic rings; it has been shown to induced superoxide formation in liver microsomes and submitochondrial particles (Thayer, 1977).

It was therefore postulated that adriamycin might influence oxidation-reduction reactions in tissues and induce damage through free radical formation. Indeed, in experiments reported by Myers and coworkers, (1976, 1977), pretreatment of mice with α -tocopherol—an effective free radical scavenger known to inhibit the formation of lipid peroxides—greatly reduced the toxicity associated with adriamycin treatment, without affecting its antitumoral activity.

We have detected the formation of lipid peroxides by measuring the production of malondialdehyde (MDA) in heart tissue of normal CDF₁ mice given single i.v. doses of adriamycin (5-30 mg/kg b.w.). With all the doses used, a short-lived increase in heart MDA production was observed 3-4 days after treatment, in concomitance with maximal decrease in total body and heart weight of the animals (Delaini *et al.*, 1978). This biochemical effect was not dose-dependent in the range of doses used and did not appear in three other different strains of mice tested. Treatment of CDF₁ mice with α -tocopherol (85 U/mouse i.p.) 24 hours prior to adriamycin administration, resulted in inhibition of heart MDA production, without correcting either the survival time, or the body and heart weight loss of the animals. Therefore, the normalization by α -tocopherol treatment of heart MDA, a biochemical parameter representative of lipid peroxidation, was not accompanied by reduced general toxicity.

It is conceivable that in our experimental model enhancement of lipid peroxidation in the heart did not represent the main mechanism of toxicity, or, otherwise, that the scavenging function of α -tocopherol, was not sufficient to prevent some free radical formation which went undetected by our MDA assay.

Cells and tissues are protected against damage by oxidizing free radicals through a complex battery of antioxidant mechanisms, which are closely interrelated (Dormandy, 1978). These include plasma protein components (caeruloplasmin, transferrin, haptoglobins) and antioxidant enzymes (superoxide dismutase, catalase, glutathione-peroxidase). The latter are widely and differently distributed in various cells and tissues; the low catalase content in heart tissues compared to other organs (liver, kidney) has been implicated in the supposedly selective toxicity of adriamycin towards the heart (Thayer, 1977). All the superoxide dimutases so far characterizes contain in their structure one or two essential

trace metals, such as manganese, zinc and copper, the dietary intake of which can greatly vary in both laboratory animals and man.

Last but not least, selenium has long been considered an important substitute for or potentiator of α -tocopherol antioxidant activity in experimental animals (Tappel, 1962).

In conclusion, while evidence is accumulating that lipid peroxidation is an important step in adriamycin-induced cardiotoxicity, it is still difficult to obtain univocal data about the best antioxidant(s) to be used.

In view of the complexity of the systems involved, the effect(s) of antioxidant protection may be masked for a variety of reasons: the diet composition, the stage of animal development, and a number of other, perhaps still unrecognized factors. Much more experimental work is required, therefore, before clinically meaningful indications are obtained for the use of α -tocopherol in the prevention of adriamycin-induced cardiotoxicity.

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The relationship between vitamin C and prostaglandin biosynthesis

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The effect of 1-ascorbic acid on smooth muscle was studied in different systems *in vitro*. On gastrointestinal smooth muscle (rat stomach fundus strip and guinea pig ileum), this vitamin induces a dose-related contraction (Fig. 1) as does on guinea-pig myometrium during the di-oestrous phase (Puglisi *et al.*, 1978). An opposite effect, i.e., relaxation is elicited, by this small molecule, on guinea pig tracheal chain preparation and on human non-pregnant isolated uterine strip. These effects show a similar pattern to that of prostaglandin (PG) of the E type on the same preparations.

The mechanism of this pharmacological action appears to be mediated through the release of another biologically active compound. The contraction or relaxation is indeed blocked only by indomethacin (Fig. 1) or by 5, 8, 11, 14-eicosate-

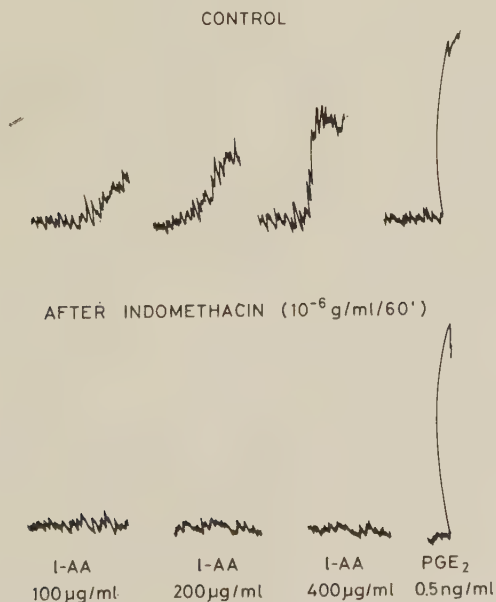


FIG. 1. Effect of 1-ascorbic acid on rat stomach fundus strip in presence and in absence of indomethacin.

traynoic acid (ETA or TYA), two well known inhibitors of PG biosynthesis, suggesting their involvement in the 1-ascorbic acid activity.

Measurements, by mass-fragmentography (according to the method of Nicosia *et al.* (1974), of PGE_2 and PGF_2 formation and release in the perfusing medium of our different smooth muscle tissues were therefore performed. A correlation was found between the effects evoked by 1-ascorbic acid and the PGE_2 biosynthesis (Puglisi *et al.*, 1976, 1977, 1978). From these results we suggest that vitamin C may stimulate preferentially the formation of PGE_2 from arachidonic acid, acting on the two enzymes: endoperoxide isomerase (Nugteren *et al.*, 1973) and the peroxidase (Nugteren *et al.*, 1966) which are reduced-gluthione (GSH) dependent. Scurvy is associated with decreased GSH in blood and tissues (Banerjer *et al.*, 1952).

The enhancement of PGE_2 releasing due to 1-ascorbic acid may explain its prophylactic and therapeutic effect observed in respiratory diseases (Miyares *et al.*, 1973), (Zuskin *et al.*, 1973). Furthermore since also the formation of hormones such as noradrenaline, adrenaline (Blumberg *et al.*, 1965) and serotonin (Borg, 1965), together with cyclic nucleotides (Moffat *et al.*, 1972) are influenced by vitamin C it is worthwhile stressing that this vitamin will be interact and regulate, controlling or modulating, several other physiological activities.

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The effect of vitamin C on the absorption of iron

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Iron deficiency is a common nutrition disturbance from which about 10-20% of the adult population and 50% of all women in childbearing age suffer. In children and adolescent girls iron deficiency has an even higher incidence. Babies of all countries including those in Europe show depressed haemoglobin concentration which are caused primarily by iron-poor foods such as milk and cereals. In order to improve the poor iron absorption from plant products, possible absorption inducing additives have long been investigated. Of these enhancing factors only ascorbic acid (AA) is of particular interest in that a positive effect on iron absorption could be achieved, when iron was present in ferric as well as in ferrous form. In experiments with rats and healthy adults the addition of AA to iron in ferric form (NaFe-citrate or NaFeEDTA) lead to an increase in iron absorption by a factor of about 2 or 4 (Doppelfeld *et al.*, 1974; Viteri *et al.*, 1978).

In a rat haemoglobin repletion test one group received 30 ppm iron in the feed as ferrous sulphate alone, the other both 20 ppm iron in combination with 200 or 400 ppm AA. Evaluation of the measured criteria—haemoglobin concentration, weight gain, plasma iron and transferrin saturation—confirmed the null hypothesis. So the same effect could be achieved with 20 ppm iron and the addition of AA as with 30 ppm iron alone. This corresponds to a 50% improvement of iron absorption with AA.

Therapy and prophylaxis against iron deficiency symptoms can be carried out by raising the iron intake, introducing preparations with a higher iron availability or by better utilization of the iron already present in food.

For nutritional physiology the addition of absorption inducing agents is of particular interest when one deals with vegetable foods. The addition of 70 mg AA in the form of papaya fruits to a meal composed of corn lead to an improvement of iron absorption from 4.6 to 24.7% (Layrisse *et al.*, 1974).

It could also be shown by means of a double isotope red cell utilization method that the absorption of both the intrinsic food iron from a standard rice meal or from maize porridge and from an added iron compound was increased three-fold by AA supplementation during cooking (Sayers *et al.*, 1974).

The consequence of a low iron status in the mother on the sucklings could be demonstrated in an animal model. The low iron intake with mothers' milk led to a pronounced increase in lipid content of plasma and organs in the sucklings. The general rise in lipid values of babies is analogous to that found in AA deprivation (Rothman Sherman *et al.*, 1978).

In conclusion it can be said that the addition of AA to the meal has a similar effect on iron absorption as an increase of iron content in food. However the AA supplementation is more effective in increasing the quantity of available iron. For this effect it is essential that AA and iron are ingested simultaneously.

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The role of vitamin C in inflammatory diseases

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Ascorbic acid tissue concentrations have been shown to be reduced in several pathophysiological conditions including viral infections, rheumatoid arthritis and neoplasia (Wilson, 1975; Mullen and Wilson, 1975). On the basis of these observations it has been proposed that supplementary Vitamin C can exert a beneficial therapeutic effect on bacterial and viral infections. Amongst the latter most of the observations have been carried out on the common cold (Wilson, 1975). In the common cold it has been suggested that the inflammatory response is produced by viral immune complex-antibody reactions. Ascorbic acid concentrations are reduced in patients suffering from the allergic syndrome including respiratory allergic disease. It has been pointed out that the differential diagnosis between the common cold and the allergic cold is difficult (Wilson, 1975). Positive skin prick tests to pollens and house dust mite are common in children suffering from acute and chronic respiratory inflammation. This makes it likely that many of the pathophysiological respiratory inflammatory states, diagnosed as common cold, in which the therapeutic effect of supplementary Vitamin C has been tested, may have an allergic origin.

Ascorbic acid concentrations are also reduced in the autoimmune inflammatory disease of rheumatoid arthritis. In neoplastic disease, ascorbic acid is actively concentrated in the tissue involved in the neoplastic growth (Kakar and Wilson, 1975) so that the non-neoplastic tissue is deprived of ascorbic acid and approaches a condition of sub-clinical scurvy. In cancerous patients antibody control of uninhibited tissue growth is deficient. It has been suggested that ascorbic acid is selectively absorbed by the neoplastic cells in order to enable their invasive growth, and by the host cells in order to potentiate their immune resistance to the invading cells. The more intense the inflammatory reaction round the neoplastic growth the greater the requirement of ascorbic acid.

Ascorbic acid plasma and leucocyte concentrations are reduced, and local tissue concentrations are raised, in inflammatory reactions of infective, neoplastic and allergic origin. Ascorbic acid plays an important but varied role in these inflammatory responses. Evidence for its direct action in an allergic inflammatory response has been demonstrated by applying ascorbic acid to

aphthous ulcers. When challenged by the specific allergen responsible for their development, pain and inflammation occur in these ulcers. Prior local application of ascorbic acid prevents the development of this inflammatory reaction (Wilson, 1978).

The interaction of aspirin with ascorbic acid in inflammatory states

Ascorbic acid is bound to ovarian protein (Sharma and Wilson, 1978) to plasma (Manhanram *et al.*, 1970), and to serum albumen (Tuckamoto *et al.*, 1974). Specific and non-specific binding sites for ascorbic acid to serum albumen have been shown to exist. Addition of acetyl salicylic acid to the medium reduces the number of binding sites for ascorbic acid to the serum albumen (Lambert, Molloy and Wilson, 1977).

It has been shown that administration of aspirin with a loading dose of ascorbic acid to normal human beings prevents elevation of leucocyte ascorbic acid. Plasma ascorbic acid falls and urinary excretion increases so that a state of sub-clinical scurvy is approached. In contrast, administration of aspirin together with supplementary Vitamin C during the common cold increases leucocyte uptake of ascorbic acid. Aspirin potentiates the ability of leucocytes actively to take up ascorbic acid while the patient is suffering from the inflammatory respiratory reaction (Wilson and Greene, 1978). Comparison of the uptakes of ascorbic acid into the leucocytes of normal and rheumatoid patients demonstrates that aspirin and ibuprofen also cause a significantly greater uptake in the rheumatoid patients (Mullen and Wilson, 1975). The uptake of ascorbic acid by leucocytes from allergic subjects is reduced in the presence of the allergen to which they are sensitive (Wilson *et al.*, 1975). This effect is inhibited when aspirin, ibuprofen or sodium cromoglycate is added to the incubation medium. The leucocyte uptake of ascorbic acid is restored to the normal value, comparable or greater than that found when the leucocytes are incubated in ascorbic acid alone. The administration of aspirin to patients suffering from asthma, in whom leucocyte ascorbic acid concentrations are reduced, has been shown to exert significant beneficial therapeutic effect in reducing the effects of the antigen-antibody reaction (Collier, 1969).

These results in the common cold, in rheumatoid arthritis and in the allergic reaction indicate that aspirin interacts with ascorbic acid in patients suffering from an immune response. Aspirin makes more free ascorbic acid available in the plasma by causing its release from binding sites on the plasma albumen. Its anti-inflammatory effect appears to be exert through its action in enhancing the uptake of ascorbic acid by cells subjected to the antigen-antibody reaction.

The action of ascorbic acid in the inflammatory immune response

Ascorbic acid exerts a major role in the stimulation and control of the humoral and cellular responses to antigen stimulation. It controls and causes increased synthesis of IgA, IgM and C3 complement component as a response of the humoral defence system (Prinz *et al.*, 1978). It has been shown that ascorbic acid acts as an interferon inducer which then promotes cellular production of anti-viral polypeptide preventing viral replication at the ribosomal level (Siegel and Morton, 1977). Increasing evidence has demonstrated that *in vitro* lymphocyte reactivity correlates well with the prognosis in cancer patients. A significant increase in lymphocyte blastogenesis has been demonstrated in breast cancer patients following administration of supplementary ascorbic acid and it was shown that lymphocyte reactivity was significantly increased in these patients in *in vitro* studies (Yonemoto, 1978). These studies provide evidence that ascorbic acid plays an important defensive role in controlling the humoral and cellular immune response to antigen stimulation.

The raised local concentrations of ascorbic acid in the tissues where the immune reaction is taking place suggests that it is metabolically active in the cells. A universal cell response occurs to the introduction of a foreign protein, whether it be viral, allergic, or neoplastic, in the form of specific antibody fixation to antigenically stimulated cells. The antigen acts as a first messenger which activates the immune response with subsequent release of chemical mediators responsible for the defence inflammatory reaction. In patients suffering from allergic disease, or from rheumatoid arthritis, aspirin promotes the uptake of ascorbic acid by sensitised leucocytes. In cancer patients, the host plasma increases washed leucocyte uptake of ascorbic acid. This contrasts with the reduced washed leucocyte uptake in the presence of host plasma from healthy subjects. In diseases in which there is immune hypersensitivity and enhanced antigen-antibody reactions, the ascorbic acid uptake blocking activity of plasma is reduced, and anti-inflammatory drugs promote the uptake of ascorbic acid by the sensitised cells.

An inverse relationship exists between tissue levels of CAMP and the release of chemical mediators from cells involved in the antigen-antibody reaction. It is suggested that ascorbic acid plays a modulating role in the control of CAMP activity. The administration of Vitamin C to guinea-pigs is known to protect these animals from anaphylactic shock and it has been shown that Vitamin C promotes the intracellular breakdown of histamine (Chatterjee *et al.*, 1975). Histamine and ascorbic acid are therefore closely inter-related metabolically during cellular inflammatory reactions. Evidence has recently been obtained which demonstrated that the presence of histamine in lung tissue significantly

increases the production of PGE and at the same time inhibits the yield of PGF. Agents which release histamine from body tissue are also known to release prostaglandins from the cells involved in allergic reactions. Ascorbic acid and prostaglandins are also metabolically inter-related because it has been shown that ascorbic acid has a marked inhibitory effect on the yield of PGF and slightly stimulates the yield of PGE from lung tissue (Sharma, Garg and Wilson, 1976).

The metabolic interaction of histamine and prostaglandins may be classified as a defence mechanism which operates to protect lung and other tissues from over stimulation by histamine following its release during the antigen-antibody reaction. Ascorbic acid controls the formation of prostaglandins, and synthesis and rate of destruction of histamine. Ascorbic acid through its effect on noradrenaline and possibly directly can activate cyclic AMP and may affect cyclic GMP. Through these mechanisms it appears that ascorbic acid modulates intracellular function and thus controls second messenger release (Wilson, 1977).

CONCLUSION

Ascorbic acid is bound to serum albumen and tissue proteins. In disease states ascorbic acid concentrations are reduced in the plasma and leucocytes and it is actively conserved by the kidneys. In diseases involving antigen-antibody reactions ascorbic acid blood concentrations are reduced. Its uptake into the affected cells is decreased in the presence of the specific antigen, but is increased in the presence of the plasma from the diseased patient or by aspirin. Ascorbic acid enhances humoral and cellular defence mechanisms during viral attack, and in neoplasia. There is a close interaction between histamine and prostaglandin formation and their release through CAMP. Ascorbic acid is involved in this interaction and its intracellular availability controls the degree of synthesis of these two chemical mediators. It is concluded that the availability of free ascorbic acid in the plasma is a prerequisite for the control of first messenger activity in tissue immune defence and in the antigen-antibody reaction, and that its intracellular concentration determines the release of the chemical mediators which act as second messengers in the inflammatory response to antigen stimulation.

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FREE PAPERS
CARDIOLOGY, VASCULAR DISEASES

The relationship between ischemic heart diseases and catecholamines

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We made an investigation about the relationship between ischemic heart diseases and catecholamines in a series of twenty-two cases consisting of five normal persons and nine patients with myocardial infarction, two patients with angor pectoris, three patients with hypertension and three patients with chronic ischemic heart disease.

We measured diurnal urinary vanilmandelic acid excretion, a catabolic product of catecholamines, with modified colorimetric methods of Pisano and A.J. Wavod and found normal in five normal persons and ten patients with different types of ischemic heart disease. It was high in two patients with hypertension, four patients with myocardial infarction and one patient with angor pectoris.

Patients with myocardial infarction or angor pectoris having high vanilmandelic acid excretion had precordial pain when urinary vanilmandelic acid determination was made. The percentage of patients having high diurnal vanilmandelic acid excretion was 41%.

Our study suggests that precordial pain and/or psychic stress seen in ischemic diseases are the principal causes of catecholamine elevation.

Differing effects of diuretic and antiadrenergic drugs on serum lipid and potassium concentrations during the treatment of hypertension

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INTRODUCTION

Chlorthalidone, when used in the treatment of hypertension, causes an increase in serum cholesterol and triglyceride concentrations (Ames and Hill, 1976a). We summarize herein the effect of five antihypertensive regimens on serum lipid concentrations.

PATIENTS AND METHODS

The recruitment of patients, the design of the study, and our laboratory methods were described previously (Ames and Hill, 1976b). The clinical characteristics of the patients in this study are detailed in Table 1. 30 of the patients were studied on more than one of the drug regimens. The characteristics of the patients comprising the 5 therapeutic subgroups did not differ from the total group. All therapy was administered in an open, non-blinded manner.

TABLE 1

Characteristics of patients

Number		113
Male	%	53
Black-Oriental-White	%	23-1-76
Relative Weight	%	114 \pm 18*
Age	years	52 \pm 11*
Systolic blood pressure	mm Hg	158 \pm 20*
Diastolic blood pressure	mm Hg	99 \pm 12*
Serum cholesterol	mg/dl	226 \pm 41*
Serum triglyceride	mg/dl	123 \pm 65*

* Mean \pm S.D.

Measurements taken during the baseline period were averaged and subtracted from the average of the treatment measurements to obtain the difference (Δ) shown in Table 2. The 5 therapeutic regimens analyzed herein comprised diet alone and diet plus reserpine (Res) or methyldopa (MD), spironolactone (spirono), hydrochlorothiazide (thiazide), or chlorthalidone (chlorthal).

RESULTS

Table 2 shows that the diet produced a decrease in serum cholesterol. Antiadrenergic drugs caused no change in serum lipids. All diuretics induced an increase in serum cholesterol, triglyceride, or both. The potassium-wasting diuretics were associated with an increase in serum cholesterol. Blood pressure decreased on all regimens. Weight decreased only in the diet-alone and chlorthalidone subgroups.

TABLE 2

Changes in measured indexes during treatment

<i>Indexes</i>		<i>Diet</i>	<i>Res or MD</i>	<i>Spirono</i>	<i>Thiazide</i>	<i>Chlorthal</i>
Number		31	19	29	36	32
Study duration	months	9	6	9	6	6
Drug dose	mg	—	0.25-615	60	50	64
Δ Cholesterol	mg/dl	—11 ⁺	—4	+5	+13*	+12*
Δ Triglyceride	mg/dl	—1	—7	+21 ⁺	+7	+36*
Δ Potassium	mEq/L	—0.1	+0.1	+0.2*	—0.5*	—0.6*
Δ SBP	mm Hg	—11*	—11*	—13*	—14*	—24*
Δ DBP	mm Hg	—8*	—7*	—6*	—6*	—12*
Δ Weight	pounds	—3*	+1	—2	0	—4*

+ $p < 0.05$; * $p < 0.01$ vs baseline value

DISCUSSION

The average increase in serum cholesterol was small. However, cholesterol increased only in half the patients treated with the potassium-wasting diuretics (Ames and Hill, 1976a and b). In this portion of patients serum cholesterol increased 28 mg/dl. As a consequence, the probability of a myocardial infarction occurring within 6 years, as determined from tables developed from the Framingham study (Kannel, 1973), did not improve in these patients (Table 3). The patients forming Table 3 are distinguished from the total group not only by

an increase in serum cholesterol during diuretic therapy but also by a lower baseline serum cholesterol (208 vs 226 mg/dl, $p < 0.02$).

TABLE 3

Probability of myocardial infarction in six years

			<i>Before therapy</i>	<i>During therapy</i>	<i>If chol unchanged</i>
SBP*	mm Hg		157	140	140
Chol*	mg/dl		208	236	208
Probability	%	Male	5.6	5.6	4.6
(Age 50)		Female	2.0	1.9	1.6

* SBP = systolic blood pressure; Chol = serum cholesterol

SUMMARY

Serum cholesterol increases notably in 50% of hypertensive patients treated with potassium-wasting diuretic drugs, and because of this, the risk of myocardial infarction is not improved by treatment. In these patients non-diuretic antihypertensive therapy may be more efficacious in reducing the high incidence of myocardial infarction observed in hypertension.

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Nifedipine and propranolol. A double-blind comparison of therapy for angina pectoris

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SUMMARY

1. A comparison has been made between propranolol and nifedipine in the prophylaxis of angina pectoris.

2. Both drugs were equally effective.

3. Unwanted side effects were of a minor nature and no significant differences were observed between the drugs.

INTRODUCTION

Angina pectoris (Heberden, 1772) has been known to doctors for several centuries. Until the introduction of nitrites (2), no specific treatment was available and nitrites continued to be the main prophylactic and therapeutic drugs used until the value of the chronotropic effect of beta-blocking drugs in the prophylaxis of angina was reported in 1963 (3, 4, 5). A further step was the recognition that reduction of the force of contraction of the heart muscle fibres might also be of prophylactic value—an inotropic effect.

Nifedipine (6) is a drug which is known to cause reduction in the force of contraction of the heart and is believed to produce this result by impeding the passage of calcium ions either into or within the heart muscle cells (7).

We are reporting a double-blind comparison of the inotropic effect of nifedipine with the chronotropic effect of propranolol in the prophylaxis of stable angina pectoris.

PATIENTS AND METHOD

All those included in the trial had at least two months history of typical stable effort angina relieved by rest, with or without trinitrin. None had congestive cardiac failure, valvular heart disease, malignant hypertension or respiratory failure.

All other prophylactic anti-anginal treatment was stopped on entry to the trial.

Patients were allocated at random to a fixed dose of either nifedipine 10 mg three times daily or propranolol 80 mg three times daily. Treatment continued for six weeks, when the patient changed to the alternative regime. Concurrently, patients received placebo of the other drug so that the trial was double-blind and double-dummy. All patients completing the protocol took their medication correctly, as judged by tablet counting. They were allowed trinitrin only for the relief of attacks of angina. The consumption of trinitrin was used as an assessment of the efficacy of prophylactic therapy.

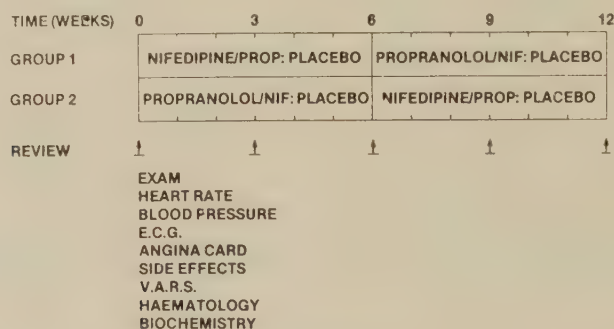


FIG. 1. Design of trial.

On entry to the trial, each patient was examined clinically; the pulse and blood pressure at rest were recorded and an electrocardiograph (ECG) was taken. A blood sample was drawn for haematological and biochemical examination.

He was then given a diary card on which to record all anginal attacks. The patient was reviewed at intervals of three weeks when the full examination was repeated. On each attendance the patient was asked to record his impressions of benefits and side effects on a visual analogue rating scale (VARS).

The ECG recordings were monitored on each attendance by the investigator and were then collected and later read in sequence by two observers who were not aware which treatment the patient had received. Any variation from the pre-treatment ECG was recorded.

RESULTS

30 patients entered the trial and 23 patients completed the protocol; 12 began first on nifedipine and changed to propranolol, whereas 11 started first on propranolol and changed to nifedipine. The main clinical features are summarised in Table 1.

TABLE 1
Details of Patients

	Group 1 <i>Nifedipine</i> <i>first</i>	Group 2 <i>Propranolol</i> <i>first</i>
Number	12	11
Age years: mean (range)	57.7 (44-69)	64.2 (57-75)
Sex: male: female	10:2	9:2
Anginal duration years: mean (range)	4.0 (0.25-13)	3.8 (0.25-9)
Previous myocardial infarct	7	2

There was no significant difference between the two groups.

The first three weeks on each drug was used as a “washout” period. Only side effects from this period are reported. All observations made in the second three weeks of treatment are reported.

The mean number of anginal attacks per patient for the second three weeks on each drug is shown in Fig. 2.

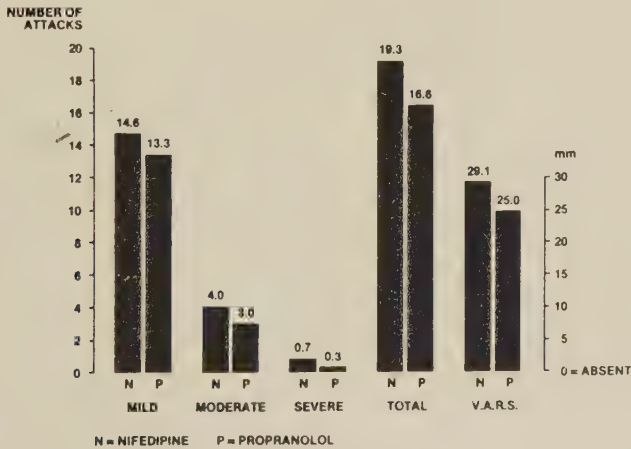


FIG. 2. Frequency of anginal attacks during trial. Mean number per patient per 3 weeks.

The attacks are divided by severity, into mild, moderate and severe and the total number of attacks is shown. The average score on the VARS is shown alongside for comparison. There was no significant difference between the frequency of anginal attacks on either drug.

The trinitrin tablet consumption (Fig. 3) reflected the frequency of anginal attacks. There was no significant difference between the number of trinitrin tablets taken on nifedipine compared with propranolol. Drug tolerance data obtained from the patient's record card and from the VARS completed at each review were remarkably similar.

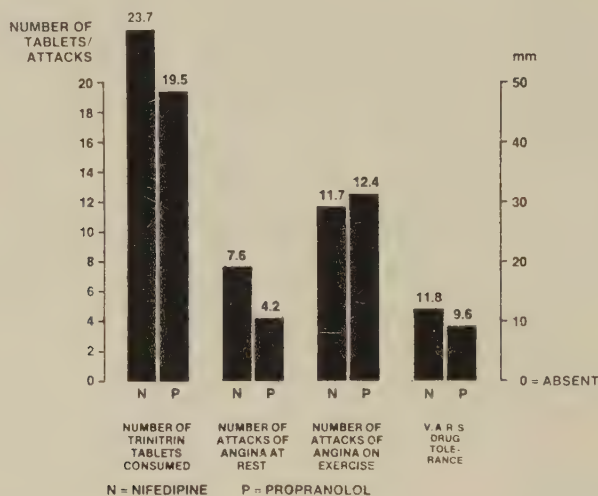


FIG. 3. Trinitrin consumption, anginal attacks, at rest and with exercise; drug tolerance. Mean values per patient per 3 weeks.

No patient developed congestive cardiac failure during the trial. The ECG's of 16 patients remained unchanged throughout the 12 weeks. The ECG's of 17 patients varied during the trial, but no constant pattern of change was seen (Table 2).

The heart rate at the end of nifedipine therapy had increased by a mean of 8 beats per minute ($p < 0.025$) compared with pre-treatment. At the end of propranolol treatment, there was a fall in heart rate of 9 beats per minute ($p < 0.001$) compared with pre-treatment. Both these changes were significant.

The blood pressure fell on both drugs. With nifedipine the mean change in the systolic level was 11.3 mm Hg ($p < 0.05$) and the diastolic 3.7 mm Hg. On propranolol the systolic level fell 5.7 mm Hg and the diastolic 7.4 mm Hg ($p < 0.001$).

The haematological and biochemical data showed no changes which could be attributed to the drugs.

14 patients complained of minor adverse effects on nifedipine compared with 15 patients complaining of minor adverse effects on propranolol (Table 3). The

TABLE 2

ECG changes of 7 patients, who showed alterations during the trial

<i>Patient No.</i>	<i>Pre-treatment ECG</i>	<i>First 3 weeks on Nifedipine</i>	<i>Second 3 weeks on Nifedipine</i>	<i>First 3 weeks on Propranolol</i>	<i>Second 3 weeks on Propranolol</i>
5	Ischaemia	Improvement	No change	No change	VES*
15	Normal	Normal	Supra-ventricular ectopics	Normal	Normal
18	VES*	Normal	VES*	Normal	Normal
21	Ischaemia	Normal	Normal	Normal	Normal
9	Ischaemia	No change	No change	Improvement	No change
11	Myocardial Infarct	No change	No change	VES*	No VES*—increased ischaemia
12	Myocardial Infarct	Minor Improvement	No change	Improvement	Improvement

*VES = Ventricular extrasystoles.

TABLE 3

Number of patients completing of events during trial

<i>Event</i>	<i>Nifedipine treatment</i>	<i>Propranolol treatment</i>
Total Number of Patients	14	15
Headache	5	3
Lethargy	5	5
Flushing	4	1
Head Cold	3	2
Short of Breath	2	0
Dizzy	2	2
Weak Legs	2	0
Nausea	0	3
Miscellaneous	8	4

(Miscellaneous equals one case each of sweating, palpitation, dyspepsia, insomnia, depression, cramp, constipation and backache on nifedipine: insomnia, caldness, dyspepsia and flushing on propranolol).

number of patients reporting side effects was less during the second period of each drug treatment. There was no relationship between the side effects reported and concurrent drug therapy.

5 patients withdrew from the trial whilst on nifedipine. One developed acute bronchitis and the second died of a myocardial infarct aged 51, having already

had infarcts at the age of 34 and 40. These two withdrawals are not thought to be related to the nifedipine treatment. A further 2 patients withdrew from the trial due to increasing angina on changing from propranolol to nifedipine. This increasing angina was probably the result of abruptly stopping beta-blockade therapy and was unlikely to be related to nifedipine therapy, except in so far as nifedipine was unable to prevent the rebound withdrawal danger on stopping propranolol. This danger had not been reported (8) until the trial was almost finished. The fifth patient stopped treatment due to unacceptable headache and flushing on nifedipine.

2 patients stopped treatment whilst on propranolol, only one as a result of the drug. He felt that he was slowed up by propranolol. The other patient was withdrawn from the trial as he was not able to co-operate.

DISCUSSION

From these results, it is apparent that both propranolol and nifedipine are effective drugs in the prophylaxis of angina pectoris. No significant difference has been demonstrated between their benefits, or the frequency of unwanted side effects. It is apparent that inotropy and chronotropy both have a part to play in the treatment of patients with angina.

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Left ventricular function (LVF) in 103 patients with chronic renal failure in dialytic treatment and 1 year follow up of LVF in 67 of the same patients

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103 patients with chronic renal failure, in dialytic treatment from 1 to 10 years (short dialysis from 1972) underwent a non invasive diagnostic evaluation of left ventricular function (LVF). In predialysis conditions electrocardiogram (ECG), phonocardiograms, carotid pulse tracing and apexcardiogram (ACG) were simultaneously recorded. The following systolic and diastolic time intervals (SDTI) were measured: preejection period (PEP), left ventricular ejection time (LVET), PEP/LVET, electromechanical systole (QS_2), isovolumic relaxation period (IRP), rapid filling phase (O-F ACG), a wave (of the ACG)/ACG amplitude. Age, sex, heart rate (HR), duration of chronic treatment, diastolic blood pressure (DBP), symptomatic degree of heart failure, history of pericarditis, haematocrit (Ht), cardiothoracic index (CTI) were considered. The correlations between the above mentioned parameters and SDTI were studied.

After one year the same study was carried out on 67 of the 103 patients, pointing out changes in the cardiovascular parameters.

RESULTS AND DISCUSSION

Correlations between SDTI and the above mentioned parameters were poor of significance. DBP is directly correlated with PEP ($r = 0,365$; $P < 0,001$) and IRP ($r = 0,358$; $P < 0,005$); these results are the obvious consequence of the haemodynamic data. LVET corresponds to increased stroke volume, as known from literature (4). In our study the increase of LVET is correlated with the Ht reduction ($r = -0,334$; $P < 0,001$), in order to obtain a normal

peripheral oxygen perfusion. CTI is directly correlated with IRP ($r = 0,341$; $P < 0,005$), the result is suggestive of a reduced ventricular compliance in patients with marked cardiac enlargement.

To our opinion, the fact that SDTI were not significantly correlated with the length of the dialytic treatment, history of pericarditis and symptomatic degree of heart failure seems very important.

One year follow up of SDTI, ECG measurements and other parameters were evaluated in the second part of this study. A significant reduction of PEP was observed, LVET remained unchanged, than PEP/LVET ratio was reduced (Fig. 1). In the first evaluation PEP/LVET was increased, as in cardiac failure

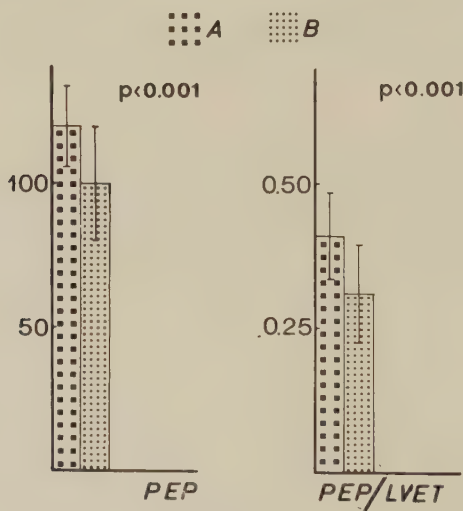


FIG. 1. PEP, PEP/LVET Mean Values \pm S.D. in the first control (A) and after one year (B).

(4), after one year PEP/LVET was reduced reaching normal values. All the other SDTI, ECG parameters, DBP, Ht, and CTI remained unchanged. One year follow up confirming unchanged clinical conditions is suggestive of the stability of cardiac parameters. PEP and PEP/LVET improvements may only mean that LVF in the year time did not deteriorate.

The aim of our study using non invasive measurements of LVF, consisted in the investigation of the role of the specific uraemic cardiomyopathy in causing heart failure in uraemic patient. Has the uraemic cardiomyopathy a single well defined cause or is the consequence of various factors such as for instance: anaemia, hypertension, hypervolemia and solutes retention? It is possible to hypothesize that in uraemic patients there is a reversible cardiomyopathy as suggested

by the improvement of cardiac condition after renal transplant, rather than an intrinsic factor responsible of cardiomyopathy. However this opinion is far to be accepted by the present literature (1, 2, 3). Our study, although limited, would exclude a progressive specific uraemic cardiomyopathy.

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Interaction of internal Na and K with respect to the kinetics of ouabain binding to the red cell membrane

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Na: K-pump and membrane-bound Na, K-ATPase are specifically inhibited by cardiotonic steroids such as ouabain. Previously it has been shown (1) that there is an inhibitory action of inside Na, $(Na)_i$, on the rate of ATP-promoted [3H]-ouabain binding to reconstituted human red cell ghosts in presence of outside K, $(K)_e$, when inside K, $(K)_i$, is low or absent. Isotonicity in these experiments was maintained with choline. Recent results of Joiner and Lauf (2), however, obtained with nystatintreated red cells, indicated that the rate of ouabain binding as well as the active K transport were both stimulated when $(Na)_i$ was increased at high $(K)_i$.

The type of result obtained by Joiner and Lauf was confirmed by our studies with red cells which contained elevated concentrations of $(Na)_i$ in exchange for $(K)_i$ after storage at 4°C for up to five days. Therefore the interaction of internal Na and K on ouabain binding was studied. For this purpose rates of ATP-promoted ouabain binding to reconstituted human red cell ghosts were followed at high $(K)_i$ keeping $(Na + K + choline)_i$ constant at 150 mM. The rate of ouabain binding, in the presence of $(K)_e$, was stimulated by increasing $(Na)_i$ up to 50-75 mM in exchange for $(K)_i$. Further increases of $(Na)_i$ accompanied by decreased $(K)_i$ inhibited ouabain binding as observed previously. The onset of this inhibitory action on ouabain binding is shifted to lower concentrations of $(Na)_i$ as the level of $(K)_i$ is decreased in circumstances where $(Na)_i$ is varied at constant $(K)_i$ keeping $(Na + K + choline)_i$ constant. Measurements of active ^{86}Rb -influx indicated that at increasing concentrations of $(Na)_i$, at all levels of $(K)_i$, the pump is stimulated, with the half-maximal concentration of $(Na)_i$ for activation being increased as $(K)_i$ is increased. Thus the mode of action of $(Na)_i$ on ouabain binding is determined by the concentration of $(K)_i$ while the Rb-transport is always activated by $(Na)_i$ under the different conditions. Increasing $(Na)_i$ increases the ouabain binding rate when $(K)_i$ is above about 40 mM/liter cells and decreases the ouabain binding rate when $(K)_i$ is below

about 40 mM/liter cells. When $(K)_i$ is approximately 40 mM/liter cells, the ouabain binding rate is unaffected by varying $(Na)_i$. These results are interpreted in terms of at least two types of binding sites for Na and K on the inner face of the pump which interact to control the accessibility of the ouabain binding site on the outer surface of the membrane.

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H.D.L. cholesterol and prevalence of coronary heart disease in Bristol, England

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Four hundred men and 100 women aged 45-64 were selected at random from age-sex registers of 16 doctors in Bristol. 71 men and 16 women had moved from their address and 46 men and 16 women declined to be studied. 283 men and 68 women attended the research clinic. Investigations included questionnaires, physical examination, an electrocardiograph and lipid studies on fasting venous blood.

Some preliminary results of plasma High Density Lipoprotein Cholesterol, total cholesterol and triglycerides are given below. When plasma HDL cholesterol level was examined by age there was no difference in either sex between those aged 45-54 and those aged 55-64. Women had consistently higher levels than men.

Plasma HDL cholesterol by age and sex

HDL Level (Mmo 1/L)	Men			Women		
	Age: 45-54	55-64	Total	Age: 45-54	55-64	Total
0.0	2	3	5	0	0	0
0.4	18	22	40	1	0	1
0.6	36	25	61	3	4	3
0.8	37	35	72	7	8	15
1.0	20	24	44	4	8	12
1.2	14	9	23	6	4	10
1.4	4	3	7	3	8	11
1.6	7	6	13	1	4	5
1.8	0	3	3	0	1	0
2.0	5	7	12	3	3	6
TOTAL NUMBERS	143	137	280	28	40	68

34% men and 65% women has levels of 1.0 Mmo 1/L or more.

Plasma HDL cholesterol (M mol/L)

	Men			Women		
	Number	Mean	(S.D.)	Number	Mean	(S.D.)
No Angina	258	0.9	(0.4)	64	1.23	(0.5)
Angina	22	0.8	(0.4)	4	1.22	(0.4)
No History MI	265	0.9	(0.4)	64	1.21	(0.5)
History MI	15	0.9	(0.5)	4	1.50	(0.8)
Normal ECG	247	0.9	(0.4)	57	1.21	(0.5)
Possible ECG	25	0.9	(0.4)	59	1.31	(0.3)
Probable ECG	8	0.9	(0.4)	2	1.45	(0.3)
No CHD	226	0.9	(0.4)	51	1.20	(0.5)
Any CHD	54	0.9	(0.4)	17	1.32	(0.3)

Plasma total cholesterol (Mmo 1/L)

	Men			Women		
	Number	Mean	(S.D.)	Number	Mean	(S.D.)
No Angina	256	5.7	(0.9)	64	6.4	(12.5)
Angina	21	5.7	(1.0)	4	6.5	(9.3)
No History MI	262	5.7	(0.9)	64	6.4	(12.7)
History MI	15	6.1	(0.9)	4	6.2	(4.4)
Normal ECG	245	5.7	(0.1)	57	6.3	(10.3)
Possible ECG	24	5.6	(0.8)	9	7.2	(19.6)
Probable ECG	8	5.7	(0.9)	2	6.1	(7.8)
No CHD	225	5.7	(1.0)	51	6.3	(11.1)
Any CHD	52	5.8	(0.9)	17	6.8	(15.2)

Plasma Triglycerides (Mmol/L)

	Men			Women		
	Number	Mean	(S.D.)	Number	Mean	(S.D.)
No Angina	256	1.55	(0.90)	63	1.25	(0.60)
Angina	21	1.86	(0.78)	4	1.89	(0.69)
No History MI	262	1.56	(0.91)	63	1.30	(0.63)
History MI	15	1.74	(0.75)	4	1.04	(0.24)
Normal ECG	245	1.57	(0.89)	56	1.26	(0.60)
Possible ECG	24	1.57	(1.05)	9	1.47	(0.76)
Probable ECG	8	1.69	(0.63)	2	1.19	(0.44)
No CHD	225	1.55	(0.91)	50	1.23	(0.58)
Any CHD	52	1.64	(0.88)	17	1.45	(0.70)

It will be seen from the tables that there is no significant difference in the levels of the three lipid fractions in either sex in those with and without coronary heart disease.

Thallium-201 myocardial scintigraphy for the diagnosis of effort angina

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Up to date, for the noninvasive diagnosis and evaluation of patients with related effort chest pain suspected to be coronary in origin, the most common and useful method is the electrocardiographic stress test. Nevertheless some patients are not identified by this test. Its diagnostic ability is based mainly on the appearance of specific electrocardiographic changes; thus it is not reliable in some conditions as abnormal electrical ventricular activation, electrolyte abnormalities or administration of drugs acting on S-T segment (5). Moreover stress test is able to reproduce chest pain in 85% (3) of the patients with effort angina and coronary artery disease confirmed by coronary angiography, and gives false negative electrocardiographic findings, in an average, in 35% of them (2, 3). On the other hand, even if rarely, especially in women (8), stress ECG is false positive.

For these reasons, frequently, there is a great need of noninvasive method for further clarification of stress test in order to reduce the number of patients undergoing coronary angiography exclusively for diagnostic purpose. An interesting method could be myocardial imaging with thallium-201, yet employed by some Authors, that needs further evaluation for correct assesment of its clinical utility (1, 2, 4, 6, 7).

Our study was carried out on 10 patients with typical effort angina, promptly relieved by nitroglycerin, by myocardial imaging with thallium-201 performed at rest and repeated during angina present at bicycle ergometer test with increasing workloads under electrocardiographic monitoring.

We performed myocardial imaging at rest after injection of 2 mCi of thallium-201; the stress scintigraphy was recorded by injecting the radionuclide during chest pain before exercise end point was reached. The electrocardiogram was monitored until it returned to its resting conditions.

In all 5 patients who developed stress electrocardiographic abnormalities, in four of them with typical aspect of coronary artery disease, in one with appearance of left bundle branch block, stress scintigraphy showed cold areas, in two of them unchanged in comparison with rest scintigraphy.

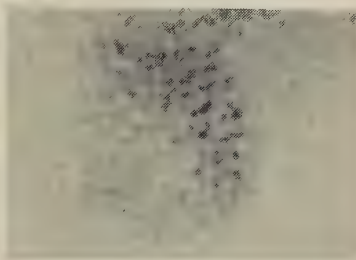


FIG. 1. Resting myocardial perfusion imaging with thallium-201, in anterior view, reveals decreased radionuclide uptake in the area referable to the septum.



FIG. 2. Same patient, same view. Stress scintigraphy with unchanged finding.

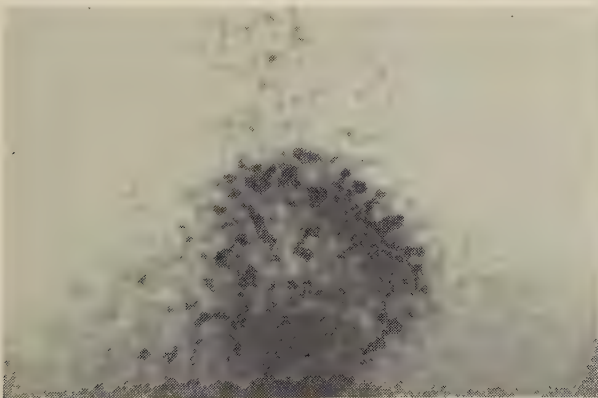


FIG. 3. Resting scintigram; anterior view. Homogeneous radionuclide uptake in whole myocardium of left ventricle except for the apex.

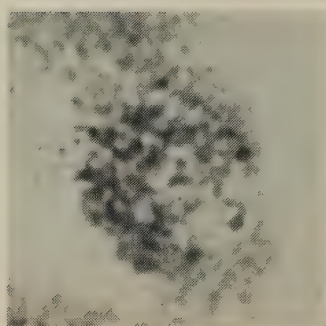


FIG. 4. Same patient, same view. Stress Tl-scan reveals cold area in the anterior-lateral wall and in the apex.

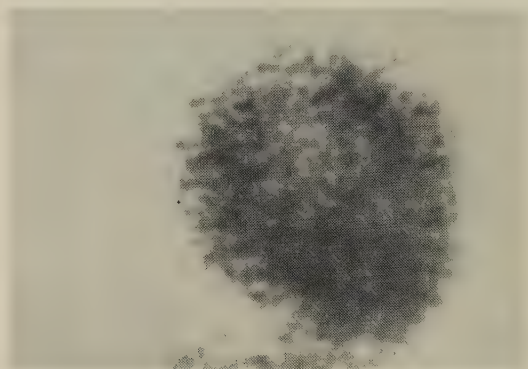


FIG. 5. Resting Tl-scan in anterior view. Homogeneous radionuclide uptake in whole left ventricular myocardium.

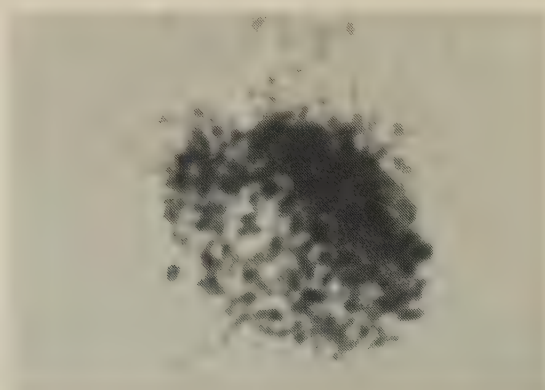


FIG. 6. Same patient, same view. Stress scintigraphy reveals a drastically decreased radionuclide uptake in the area referable to the interventricular septum.

In 2 patients with normal ECG during effort angina that became positive for ischemia only during recovery period, stress scintigraphy showed cold areas. In 2 patients, one of them affected by obstructive arterial disease of the legs, with always normal stress ECG, stress scintigraphy showed appearance of cold areas.

In 1 patient, in spite of symptoms, stress ECG, stress scintigraphy and coronary angiography were normal.

Myocardial scintigraphy with thallium-201, as indicator of relative myocardial perfusion, exhibits left ventricular normal myocardium by an homogeneous uptake of radionuclide that appears drastically increased at stress scintigraphy. In contrast regional impaired coronary supply because of disomogeneous blood flow in the whole myocardium is visualized in thallium-scan as presence of cold areas.

TABLE 1
Subjects with effort chest pain investigated by Thallium-201 myocardial scintigraphy

<i>ECG stress test</i>	<i>Patients</i>	<i>Rest scintigraphy</i>	<i>Stress scintigraphy</i>
<i>Appearance of abnormalities during exercise</i>			
— in 4 patients with typical ischemic changes of S-T segment	3	Homogeneous uptake	Appearance of cold areas
— in 1 patient with appearance of left bundle branch block	2	Presence of cold areas	Unchanged
<i>Appearance of abnormalities only during recovery period</i>			
— in 2 patients with typical ischemic changes of S-T segment	2	Homogeneous uptake	Appearance of cold areas
No abnormalities	2	Homogeneous uptake	Appearance of cold areas
— in 3 patients	1 normal coronary-angiography	Homogeneous uptake	Homogeneous uptake

Our investigation reveals that in patients with coronary artery disease cold areas can be observed at rest scintigraphy and remain unchanged at stress scintigraphy without meaning of prior myocardial infarction. On the other hand they can appear most commonly only during stress scintigraphy.

The aforesaid findings are more frequent than electrocardiographic specific abnormalities. In fact, electrocardiogram, in some subjects, shows specific changes only during recovery period and, in others, in spite of clinical judgement

strongly suggesting coronary artery disease, is always negative. The absence of cold areas in stress scintigraphy of our patient is in agreement with normal coronary angiography.

Thus myocardial scintigraphy seems solve some of diagnostic problems that stress electrocardiogram doesn't resolve.

Thellium-201 myocardial perfusion imaging can result complementary to stress electrocardiography when the latter is specific for coronary artery disease; on the other hand we suggest that myocardial scintigraphy performed at rest and repeated during exercise is prominent method for detecting many patients with false negative electrocardiographic findings or with equivocal or not surely definable stress electrocardiogram.

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Clinical and hemodynamic effects of intravenous disopyramide phosphate in cardiac patients

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The effects of Disopyramide on the electrophysiological properties of the heart and its action on ventricular and supraventricular arrhythmias have been recently studied. Moreover, it has been also demonstrated that disopyramide decreases cardiac output, coronary blood flow and myocardial contractility in intact dogs and decreases contractility in isolated perfused rabbit hearts. It has been also shown that in normal subjects doses of this drug which would prevent arrhythmias do not induce depressant effects on contractility nor on others hemodynamic factors, while they might cause alterations in patients with heart diseases. Thus, it seems likely that the depressant effects of disopyramide are most marked whenever ventricular myocardium is severely impaired.

Accordingly, the goal of this study was on one hand to further evaluate the antiarrhythmic properties of disopyramide, and, on the other hand, to assess the effects of this drug on cardiac inotropism in patients with heart diseases in different functional classes of the NYHA.

Thus, 29 patients admitted in our coronary care unit for ischemic heart disease, who developed different types of arrhythmias, received 1,5 mg/Kg of body weight of disopyramide intravenously over a period of 5 min.

In 6 out of 8 or 75% of patients with supraventricular tachycardia, disopyramide was able to interrupt the arrhythmia within few minutes following the administration of the drug.

One of these cases is illustrated (Fig. 1). On the bottom, it is shown a supraventricular tachycardia with retrograde atrial activation, which is interrupted 7 min after the intravenous administration of 100 mg of disopyramide. On the top, the standard 12 Leads electrocardiogram of the same patients is shown.

Another case of supraventricular tachycardia with aberrant intraventricular conduction (Fig. 2). Heart rate is 280 beats/min. On the right, the e.c.g. with normal sinus rhythm recorded 5 min following the administration of disopyramide.

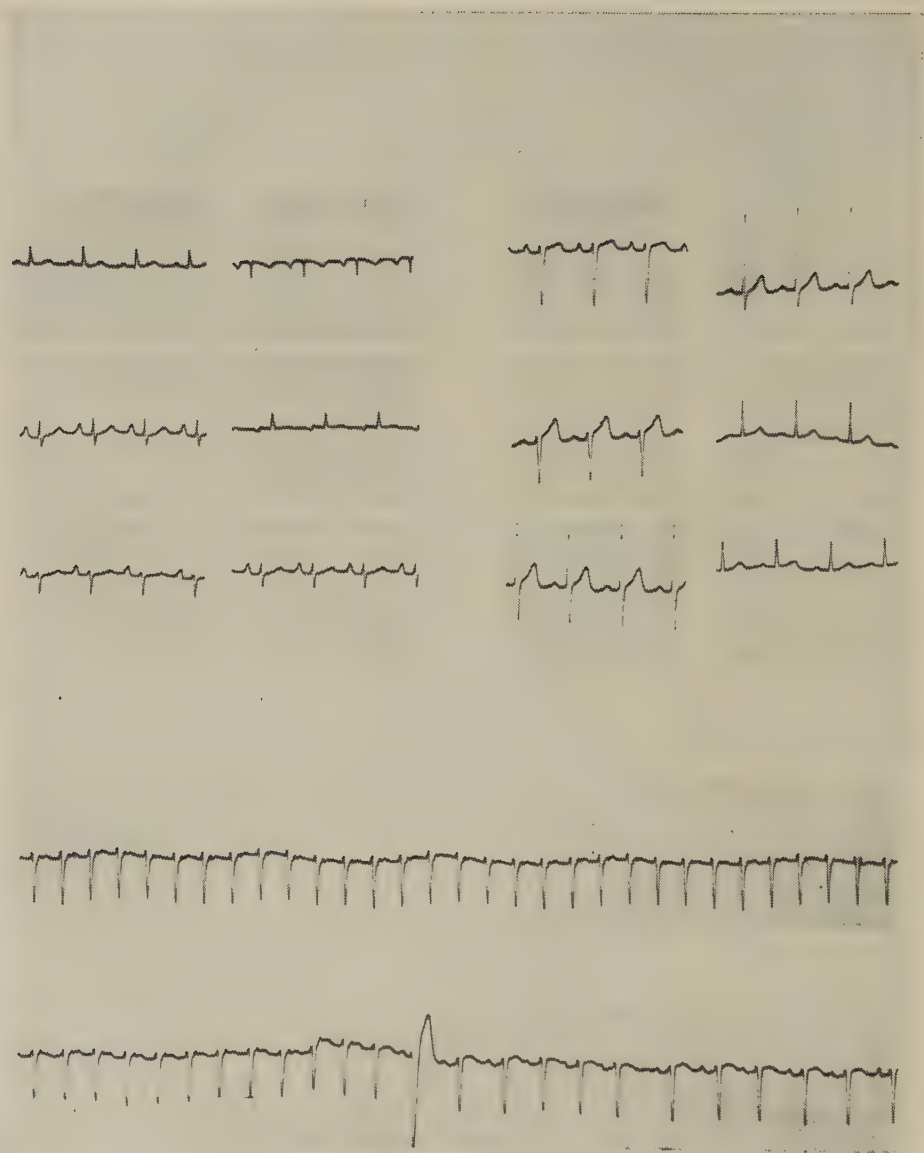


FIG. 1

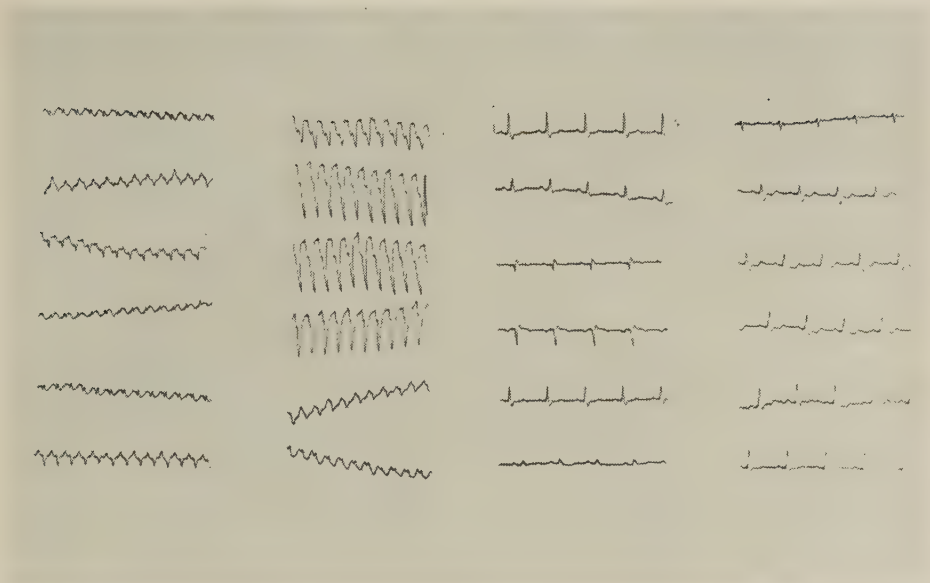


FIG. 2

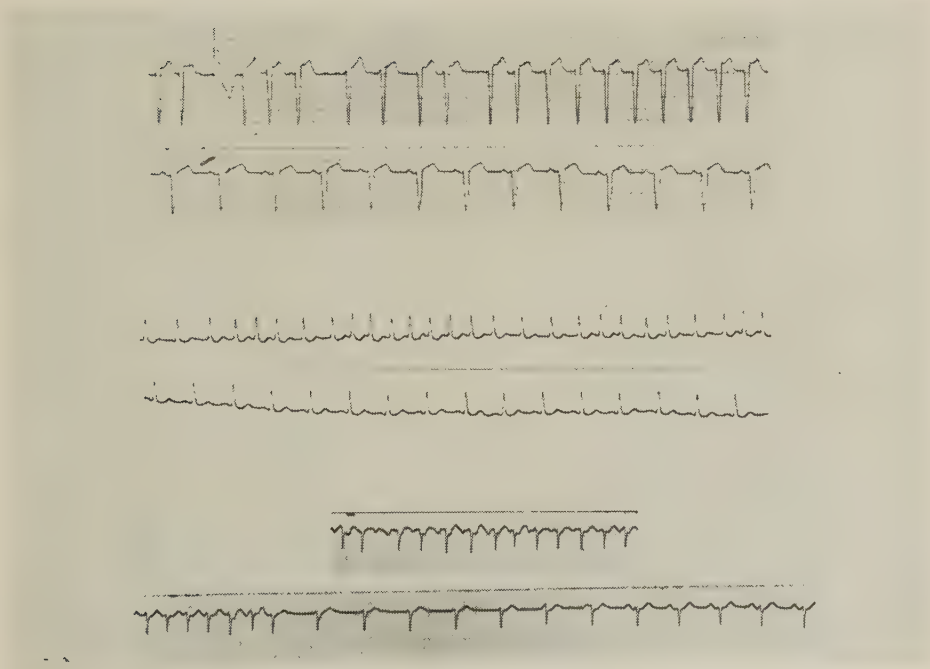


FIG. 3

Other 8 patients who received the drug were presenting atrial fibrillation with a high ventricular rate. Disopyramide interrupted the arrhythmia in 6 or 75% of these patients.

3 of these cases with atrial fibrillation at ventricular rates ranging from 120 to 140 beats/min, in whom the administration of disopyramide resulted in the interruption of the arrhythmia and the restoration of normal sinus rhythm (Fig. 3).

The drug was also given to 10 patients with sporadic, bigemini or multiple premature ventricular contractions. In 9 of these patients disopyramide was able to terminate the arrhythmia (Fig. 4).

2 of such cases, one on the top with bigemini PVCs, and the other on the bottom with multiple PVCs. Both these arrhythmias were interrupted 2 min and 3 min respectively following the administration of the drug.

The remaining 3 patients presented ventricular tachycardia and in all of them intravenous disopyramide terminated the arrhythmia (Fig. 5). In the slide 2 of these cases are illustrated. It is to be underlined that in the case showed in the bottom strips the arrhythmia was not interrupted by 100 mg of Xilocaine administered i.v., while the same amount of disopyramide restored the normal sinus rhythm.

Thus, in our experience disopyramide proved to be extremely effective in interrupting supraventricular as well as ventricular arrhythmias.

In order to assess the effects of this drug on cardiac inotropism in patients with heart diseases and the possible different actions of disopyramide in subjects with different degrees of cardiac failure, in other 15 patients with atherosclerotic heart diseases the same dose of the drug was given intravenously over a period of 5 min. Eight of the patients were classified in the 1st or 2nd class of the NYHA, while the remaining 7 were in the 3rd or 4th class. They were not receiving digitalis nor antiarrhythmics for at least 3 days prior to the disopyramide administration. Cardiac performance was evaluated by measuring systolic time intervals, which well correlate with the invasive indices of myocardial contractility. While the patients were lying in the supine position, electrocardiogram, phonocardiogram and carotid pulse were recorded simultaneously and STI were measured prior to, immediately after and 5, 10, 15 and 30 min after the administration of the drug.

The effects of disopyramide on contractility indices are illustrated in the 8 patients in I-II class and in the 7 patients in III-IV class. It can be observed that the administration of the drug resulted in a significant increase of isometric contraction time (on the top), pre-ejection period (showed in the middle panel) and of the PEP/LVET ratio (in the bottom panel), both in patients in I-II class and in those in III-IV class. However, in these latter the rise seems to be much

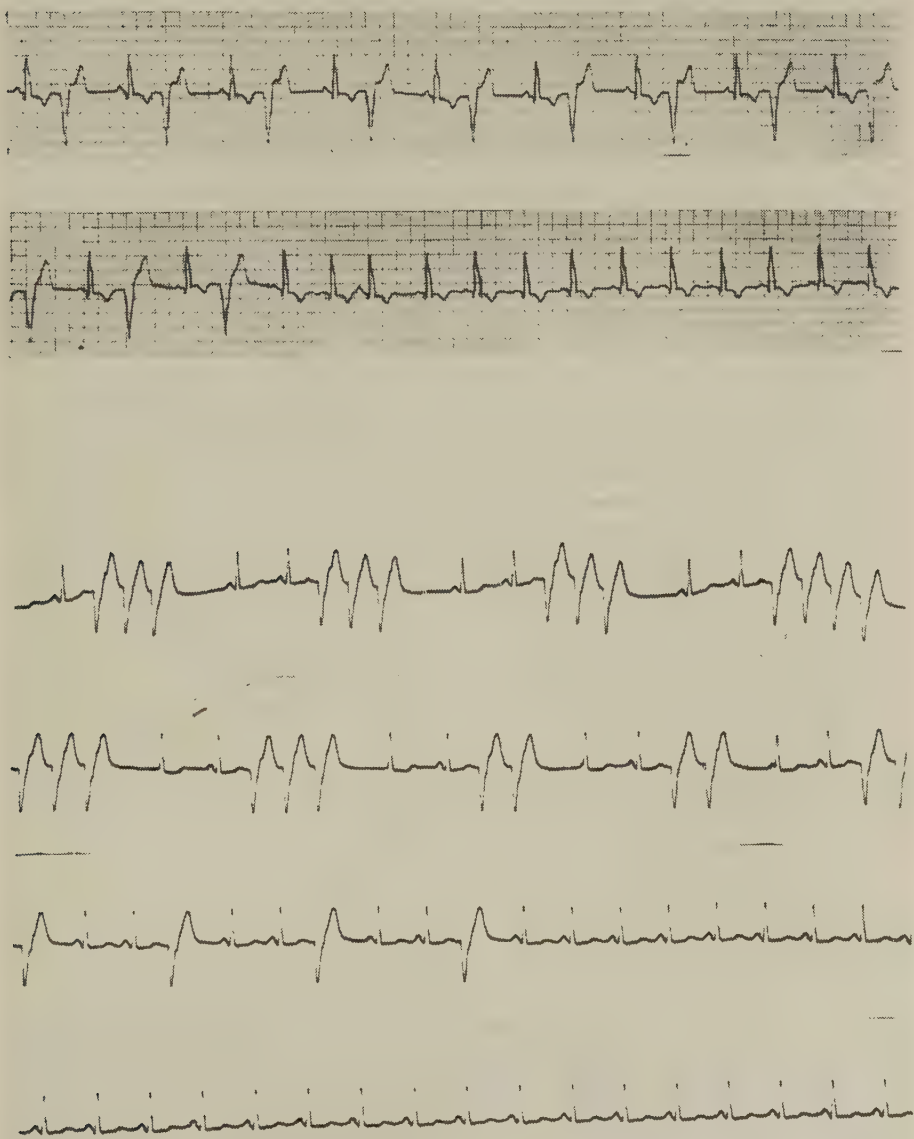


FIG. 4

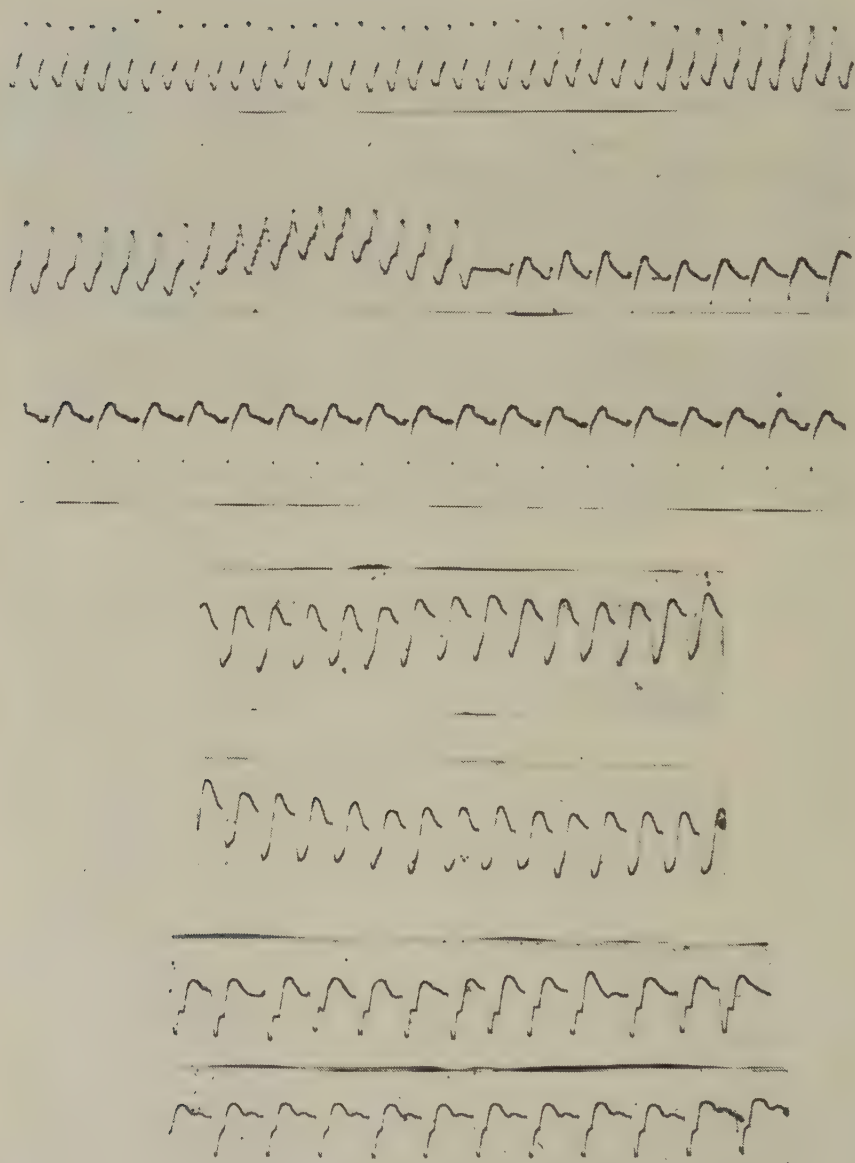


FIG. 5

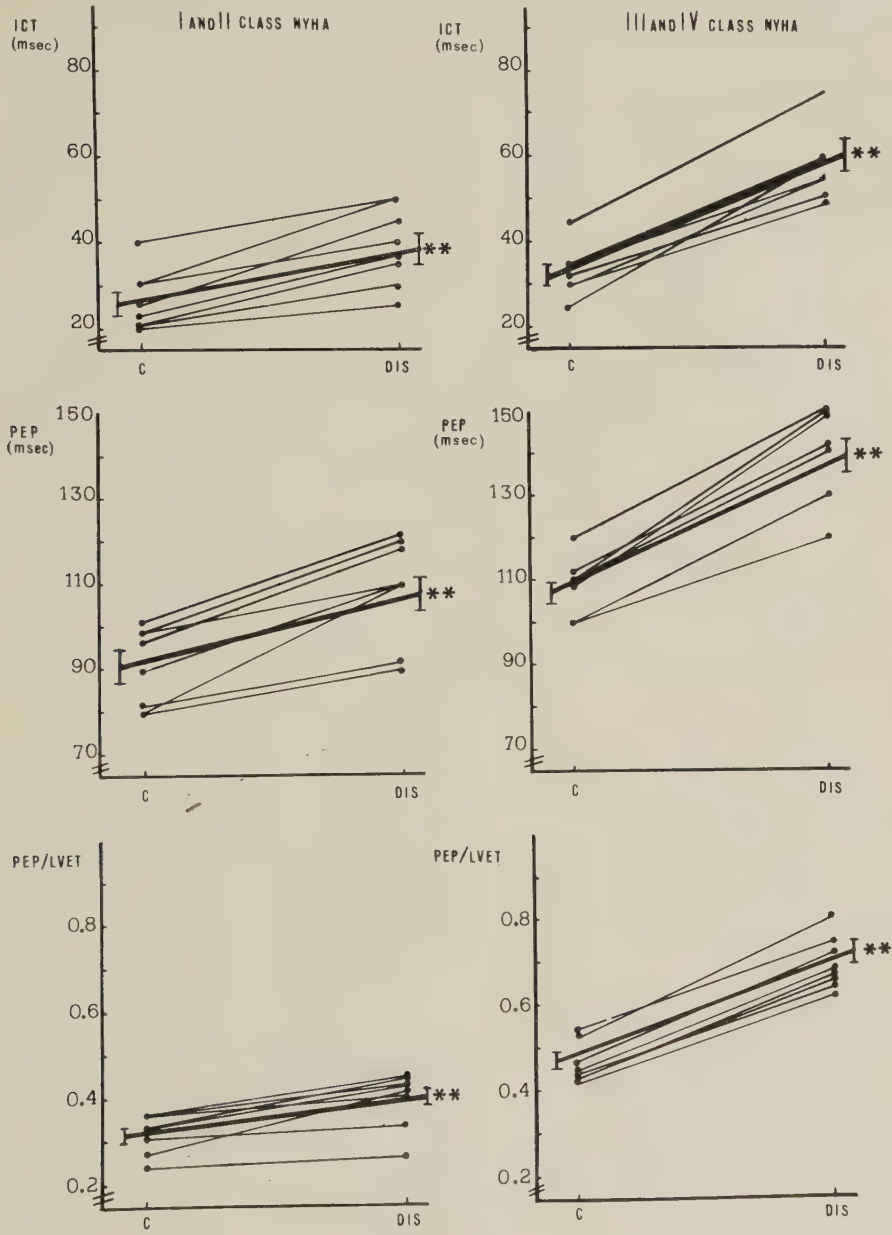


FIG. 6

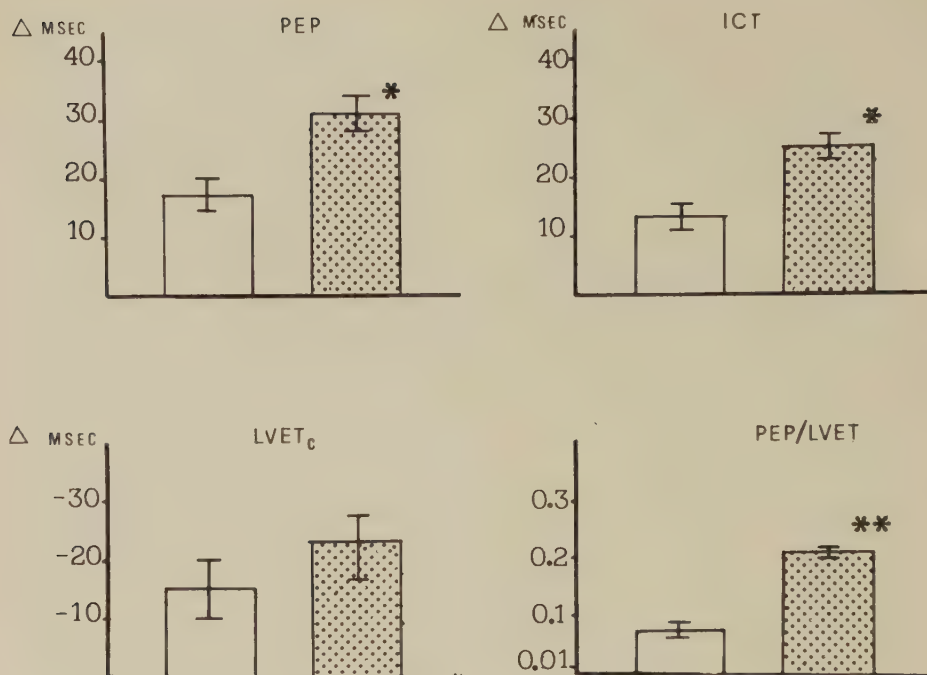


FIG. 7

more marked. Left ventricular ejection time corrected for heart rate was also affected by the drug and was reduced slightly though significantly in patients in I-II class, while in patients in III-IV class this parameter, which correlates with stroke volume, appeared to be more severely affected its value being reduced from 393 to 369 m/sec following the drug (Fig. 6).

The changes in systolic time intervals induced by the drug in the two groups of patients are shown in the slide. It is noteworthy that changes induced by disopyramide in patients in III-IV class were more marked than in the group of patients in I-II class, at least for contractility indices. Actually, it can be observed that the increase in PEP, ICT and PEP/LVET ratio following the drug was significantly greater in patients in III-IV class (dotted columns) as compared to those in I-II class (solid columns). In contrast, LVET_c, which correlates to stroke volume, was similarly worsened by the drug in the 2 groups of patients (Fig. 7).

The results of this study indicate that disopyramide is an effective antiarrhythmic agent and that it possesses depressant effects on cardiac performance. Actually, the reduction in LVET corrected for heart rate indicates that disopyra-

mide affects negatively cardiac output. However, since the reduction of this index in the 2 groups of patients did not differ significantly, it seems likely that disopyramide reduces stroke volume almost similarly independently from the degree of cardiac failure. The drug also induced in patients with heart failure a significant increase in PEP, ICT, and PEP/LVET ratio, thus showing that it has a deleterious effect on left ventricular contractility. This negative effect appears to be more evident in patients in III-IV class, i.e. in subjects with severe impairment of cardiac function, as compared to those in I-II class, since in the formers there is a significant greater increase of these indices of cardiac contractility.

Therefore, these results indicate that disopyramide affects cardiac performance in patients with cardiac failure and that heart contractility is affected more severely in patients with heavily compromised heart function.

In conclusion, disopyramide proved to be a powerful antiarrhythmic agent effective in supraventricular as well as in ventricular arrhythmias. However, since it is likely that many patients presenting arrhythmias will also suffer for cardiac failure, the results of this study suggest that caution should be used in the treatment of arrhythmias with disopyramide in patients with severe left ventricular impairment.

Genetic aspects of mitral valve prolapse

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As mitral valve prolapse (MVP) has previously described tendend to familial occurrence, the present study was undertaken to evaluate genetic factors in the etiology of the disease.

17 propositi with MVP and 37 first degree relatives were studied by clinical examination, echocardiography and phonocardiography. Among 37 first degree relatives, MVP was found in 14 of them (37.8%). In total 31 patients studied, there was no association of ABO blood groups and the disease. Compared to 302 healthy controls, HLA-B8 and HLA-B18 were found in significantly higher frequency in patients suffering from MVP (29% and 25.9% respectively) than in healthy controls (13.9% and 14.9% respectively; $p < 0.05$; relative risk = 2.4 and 1.99 respectively). Chromosomal study revealed no pathological finding. Dermatoglyphic analysis was done by studying quantitative traits (TRC, PII, a-b, b-c, c-d ridge counts and atd angle) and qualitative properties of digito-palmar dermatoglyphics (arch, loop, whorl and presentation of the patterns in the region of thenar and hypothenar) and results presented in comparison with the normal population.

Pedigree analysis, suggesting autosomal dominant mode of inheritance, and association with some HLA antigens strongly indicate the genetic predisposition to MVP.

Repercussions of the vascular encephalic accidents of the cardiovascular apparatus

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In the clinical observation on interned patients performed in the Medical Clinical Service with cerebralvascular accident of different etiology, evidences show that changes are produced in the hemodynamic conditions in the acute period of the process and that in many cases causes death. Then it overcomes a stage for attendance to the patient during recovery. Two hundred patients are analyzed with A.C.V.

1. Etiology: frequency due to age. Unchainable factors.
2. Antecedents.
3. Clinical signs.
4. Electrocardiographic changes.
5. Radiology and laboratory.
6. Physiopathogenic interpretation. Consideration on the synthesis of different mediator substances on the base of central nervous system.

Cardiac involvement in Duchenne's muscular dystrophy (D.D.B. 1)

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Duchenne's muscular Dystrophy is the more frequent congenital muscular disease. In France about 20,000 cases are recorded. Muscular involvement begins early in the childhood; progressive thoracic deformities and pulmonary infection are the main cause of death in the most evolutive form (D.D.B. 1). Congestive heart failure is present in some patients. This work's purpose is to show,

— first: that myocardium is involved as well as peripheral muscle in the early stage in patients without clinical heart failure,

— and secondly: that a relationship occurs between the severity of cardiomyopathy and peripheral muscular status.

Fifteen patients were investigated. The youngest is twelve years old, the oldest is twenty one. All have typical electrocardiographic pattern. None but one experienced clinical congestive heart failure. Invasive studies are performed with right and left heart catheterization: left ventricular systolic pressure (L.V.S.P.); L.V. enddiastolic pressure (L.V.E.D.P.), cardiac index (C.I.) and contractility index (V.E.C. max c/s) are recorded. Enddiastolic volume (E.D.V.), Ejection fraction (E.F.), mean velocity of circumferential shortening of fibers (V.C.F. c/s) are calculated with a computer from L.V. cineangiography frames (R.A.O. 30° view).

According to these later parameters, three groups of patients are separated. Group I of seven patients with normal values ($EF \geq 0.60$; $VCF \text{ c/s} > 1$; $EDV < 100 \text{ ml/m}^2$); group III of four patients with obvious hypokinetic cardiomyopathy ($EF < 0.40$; $VCF \text{ c/s} < 0.6$; $EDV > 130 \text{ ml/m}^2$); group II of four others patients had intermediate pattern. Segmental wall motion abnormalities are observed in six cases. Anterior wall is involved as well as postero basal area. Angiographic mitral insufficiency is present in 2 cases and mitral prolapse in one other case.

Cardiac index is normal in all patients. In contrast group II filling pressures ($15.5 \pm 4.5 \text{ mm Hg}$) and group III ($18 \pm 13 \text{ mm/Hg}$) are higher than in group I ($10 \pm 5.5 \text{ mm/Hg}$) $p < 0.01$.

So, it is clear that eight patients (group III + group II) had severe or mild heart impairment. In order to reveal incipiens myocardium disorder, increased afterload was induced with Angiotensin I.V., in some patients (2 cases of group I and 2 cases in group II with mild but definite ejectional abnormalities). LVSP is raised ($\Delta 30\%$) in each case. In regard of increased LVSP, filling pressures rise (11 ± 4 mm Hg vs 20.5 ± 7 mm Hg) $p < 0.01$; systolic index decrease (63 ± 18 vs 56 ± 15 ml/m²) $p < 0.05$.

EF decrease (0.62 ± 0.12 vs 0.52 ± 0.08) $p < 0.05$, as well as \overline{VCF} c/s (1.1 ± 0.4 vs 0.9 ± 0.3) $p < 0.05$. In contrast end diastolic volume is unchanged (102 ± 35 vs 101 ± 38 ml/m²).

These data are in accordance with impaired left ventricular function.

The ultimate point is the relation between the severity of myocardium impairment and muscular dystrophy. Patients are separated according to their muscular status (i.e. confinement in wheelchair and thoracic deformity). Group I patients had no thoracic deformity (but one); in contrast 6/8 patients of group II and III had mild or severe cyphoscoliosis. Similarly 6/8 patients in wheelchair since more than three years had hypokinetic cardiomyopathy; 6/7 patients with recent muscular involvement had no evidence of heart disorder.

In summary, haemodynamic and angiographic studies reveal cardiomyopathy in 8/15 patients with D.D.B. 1. Angiotensin test reveals impaired L.V. function in 2 other patients with normal values at rest. A correlation is found between L.V. impairment and muscular dystrophy severity.

Anathomo-clinical contribution to the classification of myocardial pathology apparently “ primitive ”

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In a report at the congress of the Italian Society of Cardiology in 1965, regarding the etiology of the myocardial pathologies, I did specify that according with the criteria internationally adopted at that time for the classification of myocardial pathologies, the lacking of documentation of the causal agent was an element only theoretically enough to include in the group of “Myocardial pathologies of unknown origin” or “Idiopathic” or “Primitive”, according with the various dictions, the cases of myocardial pathology in which the etiology is not certain. In fact a careful exam, of the same cases, based on clinical criteria, made us come to the conclusion that, without any justification, the group of Myocardial pathologies of unknown etiology was too large compared to the group of myocardial pathologies of known etiology and reflect on clinical field a myocardial pathology polymorphous and of not univocal significance.

In the last decade it has been emphasized a useful process of critical review, of which there is a wide trace in the most recent literature, which has favoured the isolation from the group of the myocardial pathologies apparently primitive, of some morbid myocardial entities, liable of interpretation more closely related to their inner anathomo-clinical essence, even though in a presumptive prevalent way.

The subject of this report is the description of five cases of myocardial pathology observed in patients whose age was between 25 to 49 years (3 men and 2 women). The course of the illness had been characterized by recurrent episodes of cardio-circulatory insufficiency lasting from 8 months to five years. In three cases the terminal incident of congestive failure started during clinical episodes of “flu type”, developing seriously; in one case it started in the early stage of puerperium. The clinical picture was characterized by a cardiomegaly, pre-systolic gallop, 1st heart beat paraphonic, batmotropic and/or dromotropic arrhythmia (one case with tachyarrhythmic atrial fibrillation; one case with polyfocal ectopic atrial tachycardia; one case with atrial and ventricular sequence of polyfocal extrasysto-

lia and flutter 2 to 1 with subsequent transformation to atrial tachyarrhythmic fibrillation; one case with atrial parosystic tachycardia and partial atrioventricular block; one case with polifocal atrial and ventricular extrasystolia and bilateral branch block); hepatomegaly due to stasis, slight splenomegaly; dyspnoea, slight fever movement (37° - 37° , 5) in two patients, continuous remittent fever (38° - 39° , 5) in three; slight increase of VES, slight anemia, slight leucocytosis (6000-7000) with relative lymphomonocytosis, hyposideremia, hypoproteinemia with hypoalbuminemia. The radiologic picture of the heart shade was a global cardiomegaly with conspicuous increased of both diameters, longitudinal (cm 18-22-24) and transversal. There were also electrocardiographic signs of hypoxic myocardic suffering.

In all cases were present remote and recent pathological previous of repeated bacterial and/or viral infection, often to spontaneous resolution and often without appreciable clinical residue.

In four cases there was anathomo-pathologic evidence of a flaccid heart, with dark flesh, coronary artery without any lesion, valvular apparatus intact, anathomohistological picture of interstitial myocarditis of "Fiedler type" in a varied developing stage towards more or less patched fibrosis, and severe myofibril alterations; in one case, picture of "giant cells" myocarditis (post-partum myocardiopathy); in one destructive lesions including also the His bundle and both branches.

In three cases there was a repeated increasing positivity, of the complement fixation for the virus Echo (1) and Coxackie B₁ (2), but direct virologic research was negative.

In the absence of direct documentation of the pathogenous agent the cases mentioned above should be classified as myocardiopathy of unknown origin. Characteristics of clinical pictures, the course of the illness till the exitus, the anathomo-histologic report of pleiocytosis interstitial inflammation with severe suffering of the myofibril authorized instead a more correct classification as infectious myocardiopathy presumable from virus.

We underline as the missing documentation of the pathogen's noxae, only rarely authorize to speak of really unknown causes, for the difficulties that are found in the etiological research, especially of virologic agents. In such circumstances, probatory results are obtained only if the research is precocious. In case of myocardiopathy, only the demonstration of the virus in myocardic tissue acquires a valid etiological meaning, but such event is not accomplished very easily.

Magnesium-potassium metabolism and urinary aldosterone excretion following amiloride administration to patients with congestive heart failure

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Amiloride, a K-sparing diuretic, may also influence Mg metabolism. Many experimental investigations have indicated a close interrelationship between Mg and K metabolism. 10 congestive heart failure patients were studied over a 6-day period, comprising a 3-day control period involving frusemide (40 mg/day) administration followed by a 3-day test period when amiloride (10 mg twice daily) was added to the diuretic regimen. 24-hour urinary excretion of electrolytes and aldosterone were measured as were plasma Mg and K. Cellular Mg and K were assessed by lymphocyte electrolyte analysis. Amiloride reduced 24-hour urinary K excretion and increased both plasma and lymphocyte K. Plasma and lymphocyte Mg also tended to increase after amiloride administration. Amiloride resulted in a transitory reduction in urinary Mg excretion, the effect being most marked on the first day of administration. Urinary Mg returned to control values by the 3rd day of amiloride administration. Amiloride significantly increased urinary 24-hour aldosterone excretion on the 2nd and 3rd day of administration. Increased circulating aldosterone levels would tend to counteract both the K-sparing and Mg-sparing properties of amiloride and could explain the transitory nature of amiloride's action in reducing urinary Mg excretion.

Supported by the Medical Research Council of Ireland.

“The importance of the non-invasive evaluation of myocardial O_2 consumption (MVO_2) in coronary artery disease treatment”

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The main parameters affecting MVO_2 are: 1) systolic stress of the ventricular wall, related to *a*) the intraventricular pressure and *b*) the volume; 2) the ventricular wall systolic stress period, in relation to *a*) heart rate and *b*) systolic time intervals; 3) myocardial contractility.

The indexes commonly used for an indirect MVO_2 evaluation, such as double and triple product (1), have evident deficiencies. They do not account for two important factors affecting MVO_2 : contractility and ventricular volume. Our intention is to relate the first results of a study which considers almost all the above mentioned factors. Our method considers heart rate, arterial blood pressure and ejection time, by means of the triple product, and ventricular volume and contractility, by means of serial echocardiographic recordings.

MATERIALS AND METHODS

Ten subjects who had been suffering from stable exercise-induced angina were studied both at rest and during exercise tests on a cycloergometer, before and after chronic treatment first with propranolol administered alone (40 to 80 mg/day, for 25 days) and then with propranolol + isosorbide dinitrate (10 mg every four hours, orally administered for the next 15 days).

RESULTS

A) Beta-blocking agents treatment

1. At rest: the triple product is significantly reduced, depending on the heart rate reduction and, to a lesser extent, on the systolic pressure reduction. Ejection time is not significantly modified (Fig. 1).

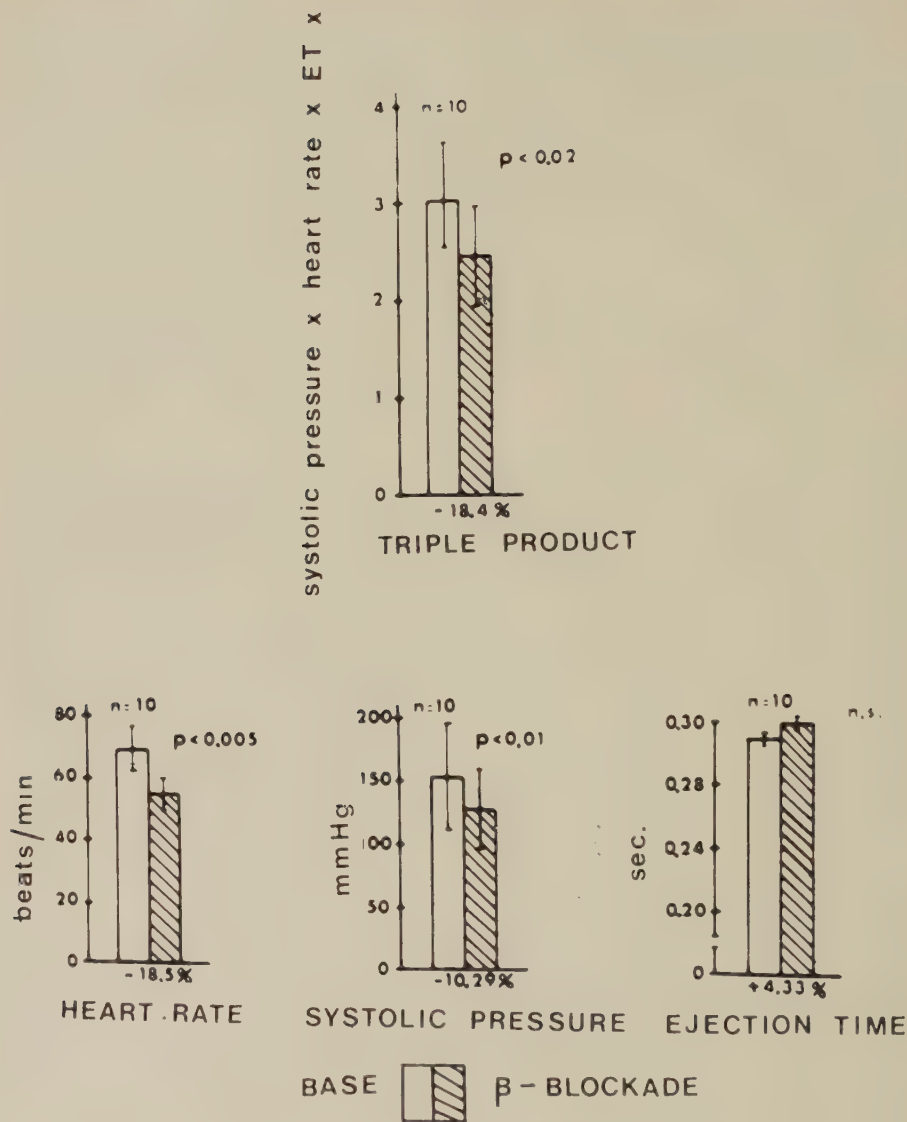
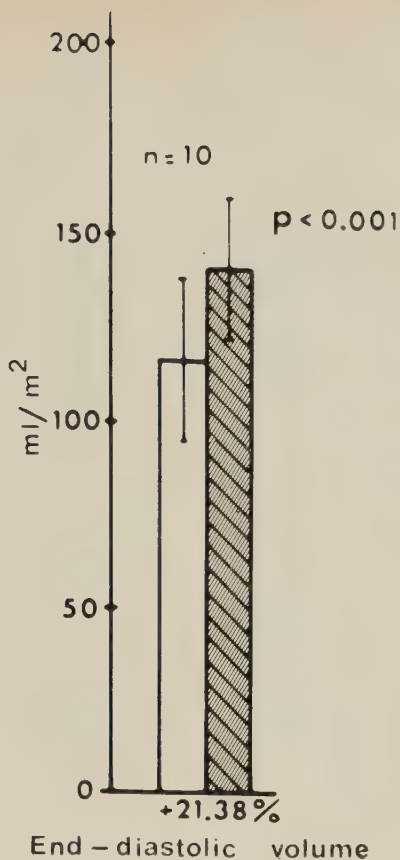
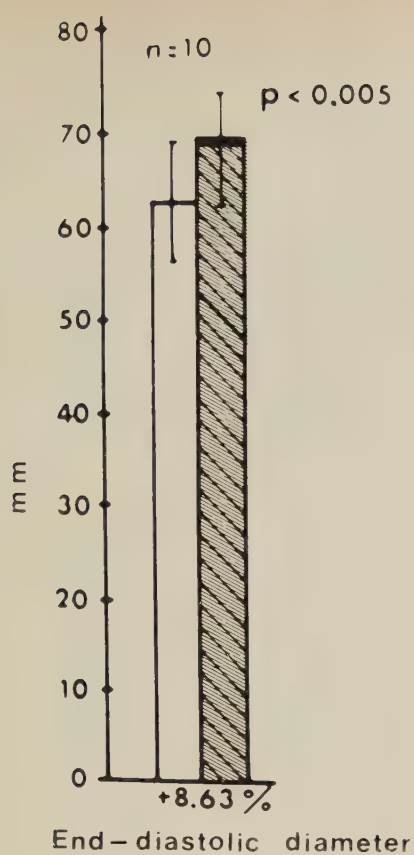


FIG. 1

Regarding the echocardiographic parameters we noticed a significant increase in the left ventricular end-diastolic diameters and volumes in all subjects (Fig. 2, 3); contractility indexes were not significantly modified (Fig. 4) except for a significant increase in pre-ejection time with a slight increase in pre-ejection/ejection time ratio:



— spontaneous changes up to 0.7 mm


BASE  BETA-BLOCKADE

FIG. 2

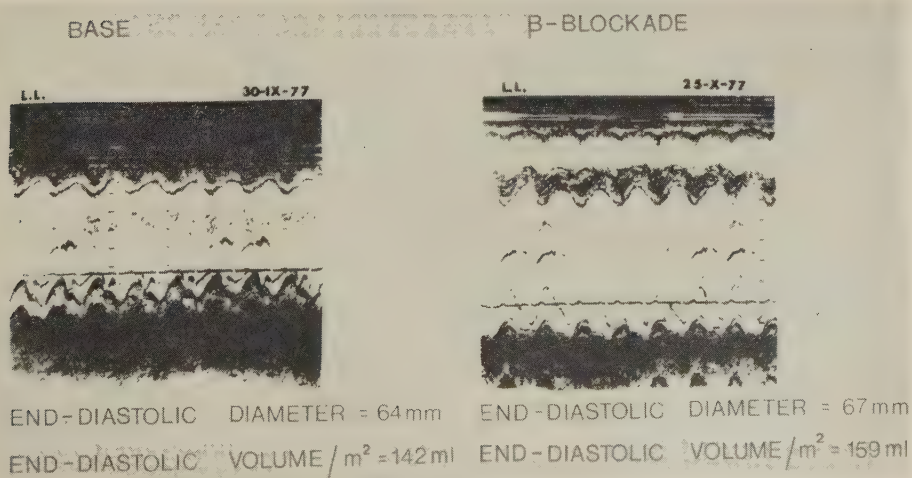


FIG. 3

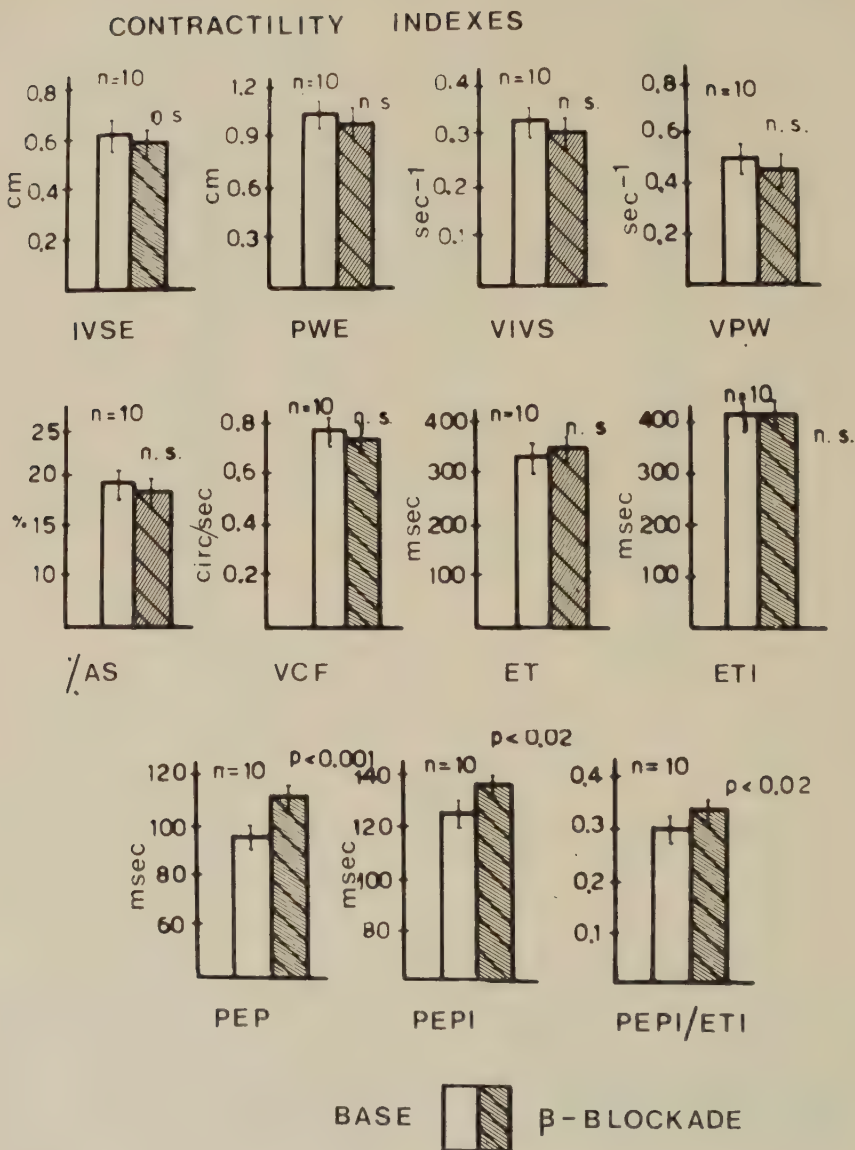


FIG. 4

2. During exercise: we noticed a marked increase in muscular work tolerance in all subjects (mean + 41.9%), although in most cases the patients stopped because of muscular fatigue, before pain. The double product is smaller than the base value (— 18.7%) (Fig. 5). The results obtained during intermediate

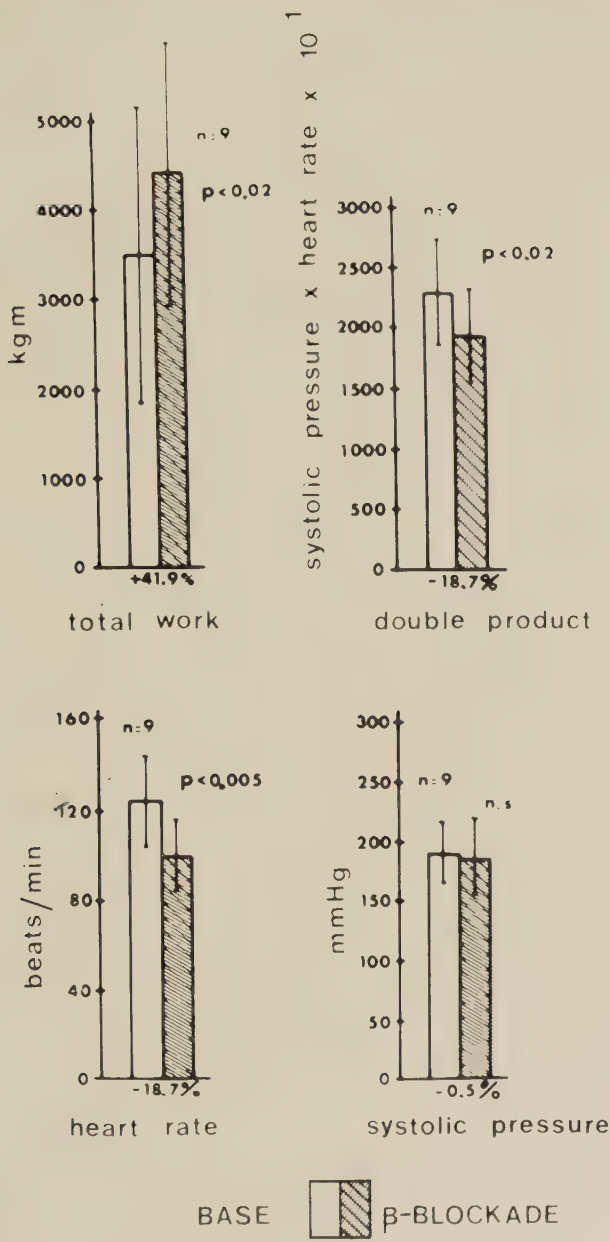


FIG. 5

degrees of exercise, i.e. not capable of inducing pain in any of the subjects examined, show that all subjects undergo exercise with a smaller double product (Fig. 6), mainly depending on a smaller increase in heart rate and, to a lesser extent, in arterial blood pressure.

INTERMEDIATE WORK

DOUBLE PRODUCT (1080 kgm)

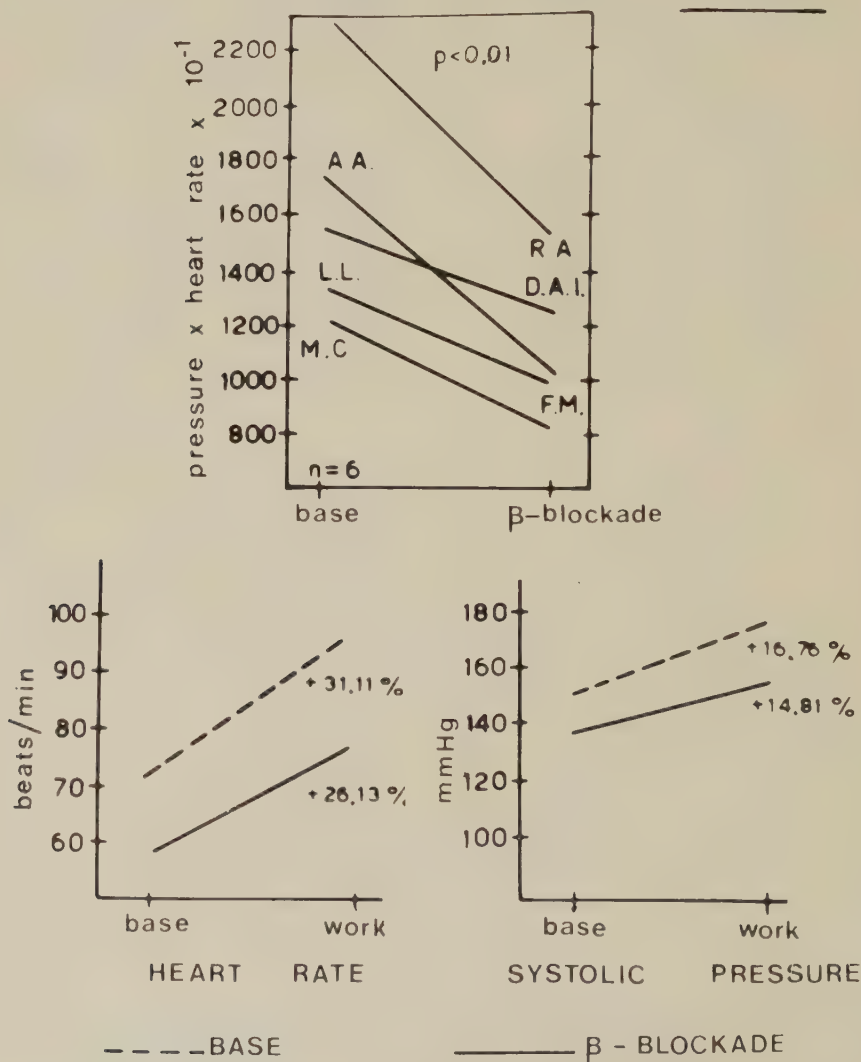


FIG. 6

One subject is worth particular attention because of the drug dose administered. In fact in this case a dose of 160 mg per day was reached and, apart from the fact that no evident modifications in heart rate and arterial blood pressure were observed, there were no improvements in exercise performance, or modifications of the triple product at rest and of the double product after muscular work (Fig. 7). On the contrary with these doses there was an increase in end-diastolic ventricular volume, the greatest increase in pre-ejection period and a PR-AC reduction under 0.06 sec.

These results show that there is a clear O_2 saving effect with propranolol at rest, mainly depending on the reduction in heart rate and, to a lesser extent,

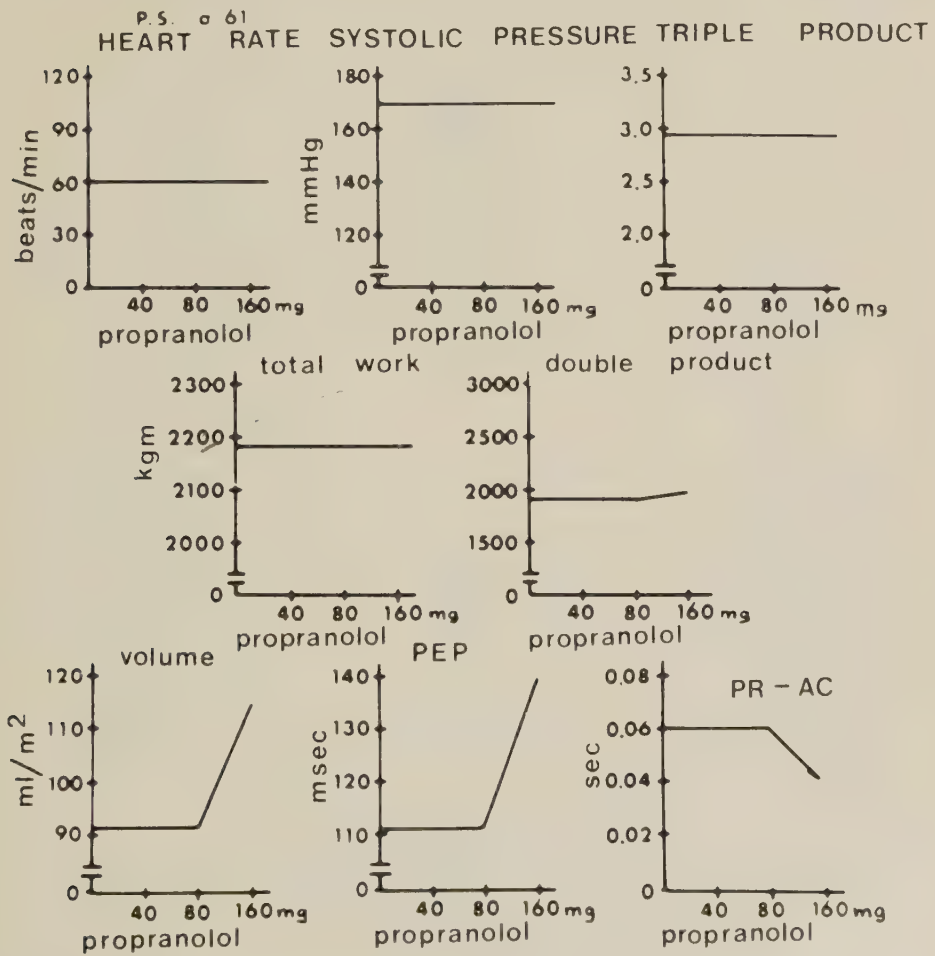


FIG. 7

in systolic arterial pressure. Contrary to other authors' observation (2-5) it is important to notice that the ejection time did not change significantly. This is likely due to the small doses we used. The results obtained show a clear O_2 saving effect with this drug under muscular work, too, as shown by greater exercise and smaller double products. In most cases muscular fatigue seems to be responsible for the fact that the patient did not arrive at the pain threshold. This could be due to the decreased myocardial contractility, but there is no clear evidence in our results; it could be, then, the consequence of a direct effect on the skeletal muscles depending on a vasoconstriction due to an alpha-adrenergic prevalence (6).

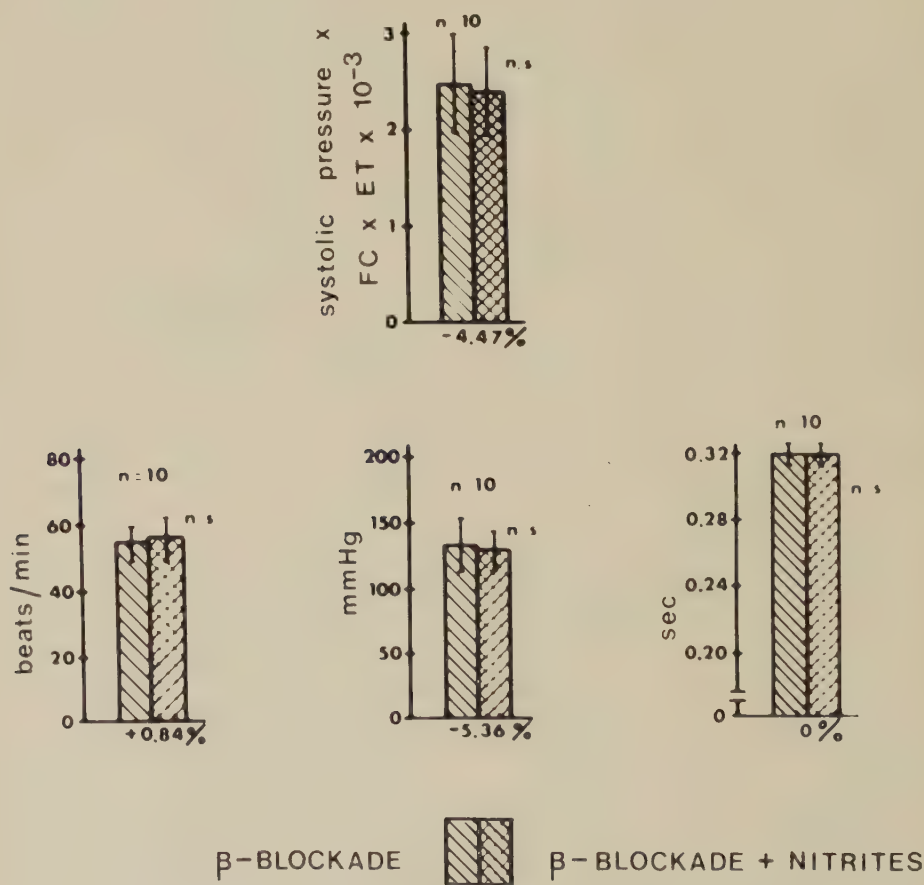


FIG. 8

B) *Beta-blockers + nitrites treatment*

1. At rest: when isosorbide dinitrate is added to propranolol no significant modifications of the triple product at rest are observed. This is due to the lack of significant heart rate, systolic pressure and ejection time variations (Fig. 8). Regarding echocardiographic parameters we found left ventricular diameters and volumes significantly reduced, tending to return to their base value (Figs 9, 10). The contractility indexes showed a significant reduction in the pre-ejection/ejection time ratio and the tendency of an increase in all the studied parameters, although the results were not significant (Fig. 11).

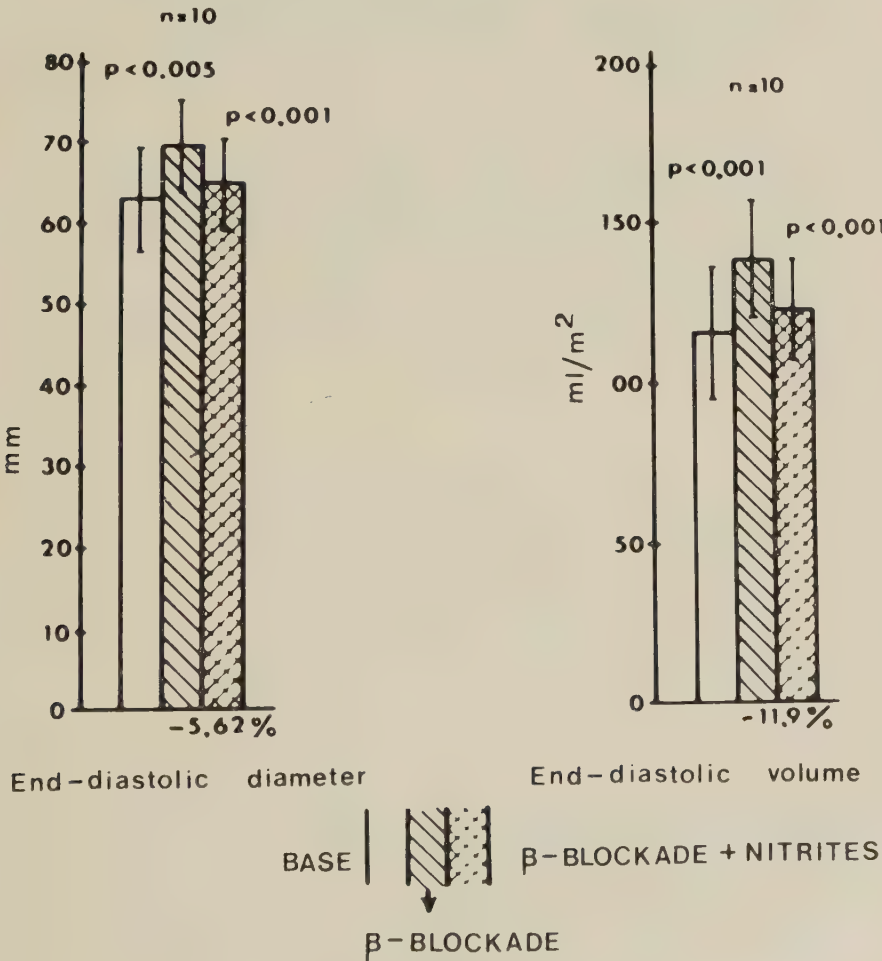


FIG. 9

2. During exercise: we found a further increase in muscular performance and double products compared to the one obtained using beta-blocking agents alone. The fact that the double product was smaller than the one obtained in base conditions, with an improved exercise tolerance, is in part due to the fact that nitrites do not remove muscular fatigue which occurs during beta-blocking

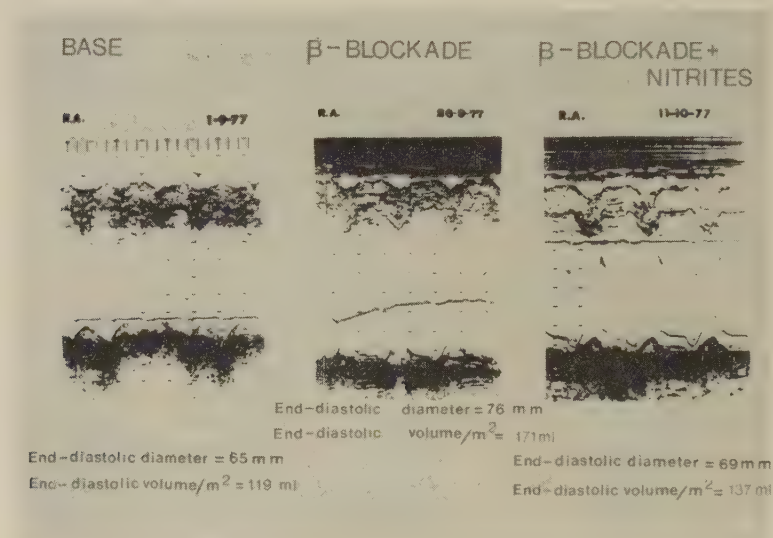


FIG. 10

treatment (Fig. 12). Finally, during intermediate degrees of exercise, the double products showed a further significant reduction, mainly depending on a further systolic arterial pressure decrease (Fig. 13). We should point out, once again, the behavior of the subject who did not show any change in the muscular performance after beta-blocking treatment but, on the contrary, showed evident muscular work (+ 56.6%) and double product (+ 12.6%) increase when nitrites were added (Fig. 14).

These findings suggest that the beta-blocking agents + nitrites produce a better O_2 saving effect both at rest (because of the decreased left ventricular diameters and volumes), and during exercise (because of an enhanced muscular performance with a lesser double product increase). The double product showed the same behavior even during intermediate degrees of exercise, depending on the further arterial blood pressure decrease.

Our results confirm that beta-blockers and nitrites are complimentary (4, 7-14). In fact, while the beta-blocking agents administered alone produce an evident heart rate and arterial blood pressure reduction with neither significant

CONTRACTILITY INDEXES

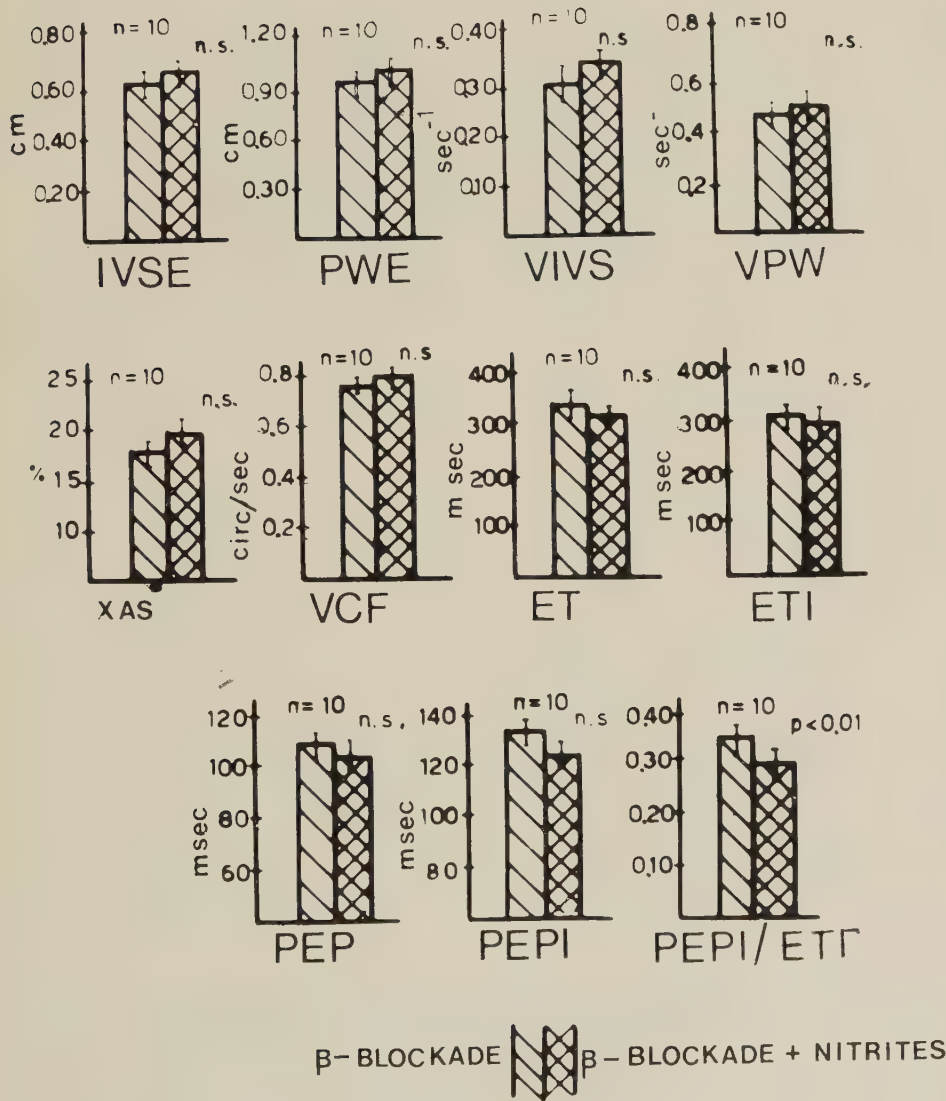


FIG. 11

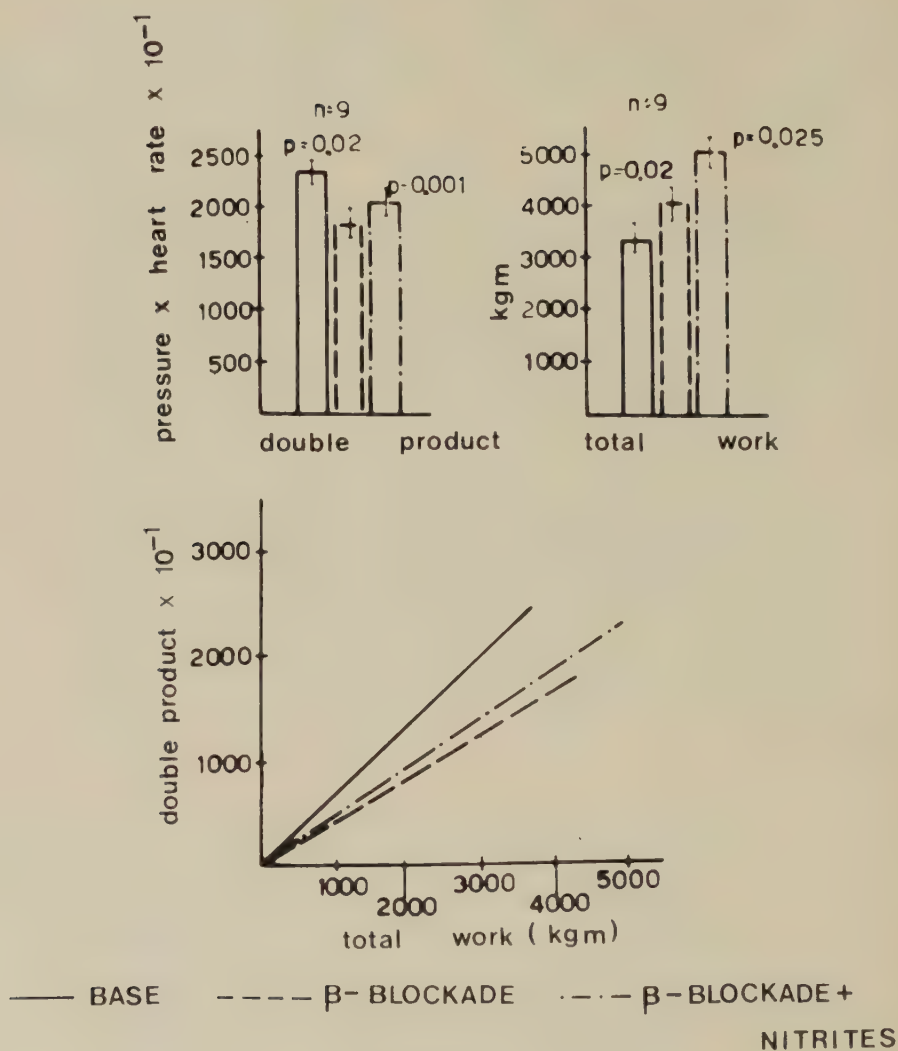
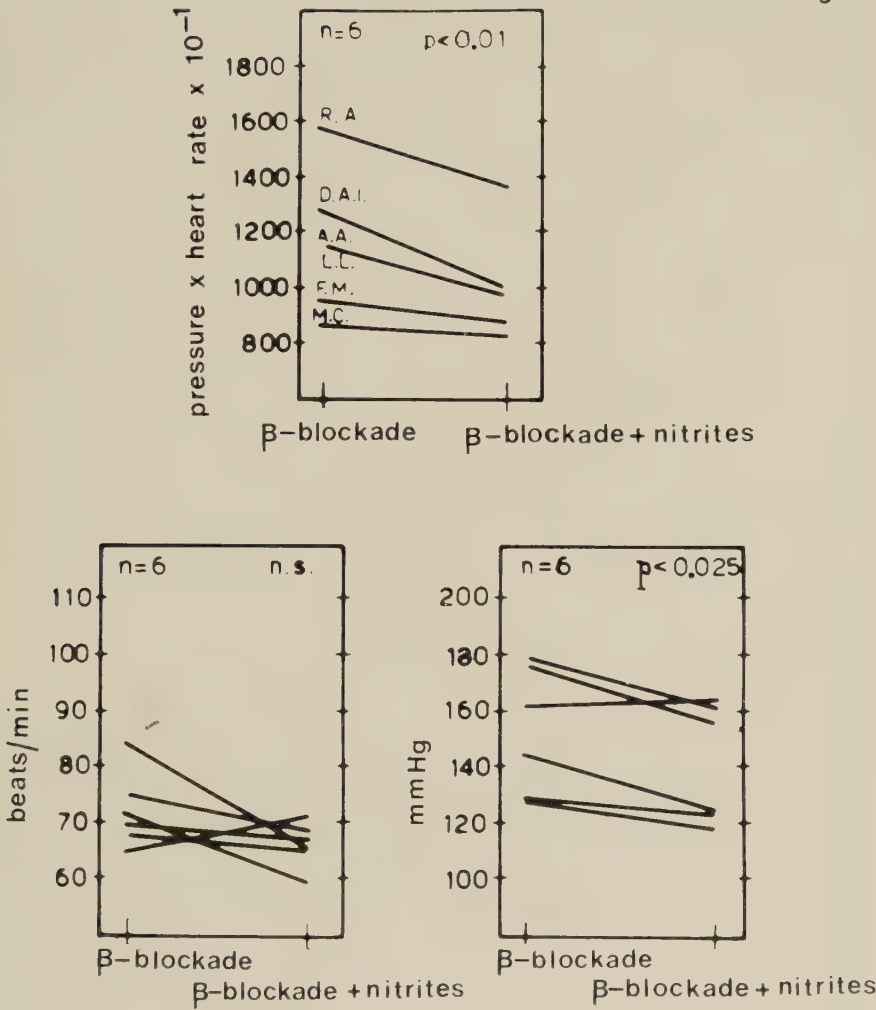


FIG. 12

ejection time modifications or contractility indexes modifications (only a slight increase in pre-ejection period), they clearly increase the ventricular volume. The MVO_2 reduction due to the arterial blood pressure and heart rate decrease, prevails over the MVO_2 increase due to ventricular volume and pre-ejection period increase. We did not find the evident hypocontractility which other authors report (2-5, 15) probably because of the small doses we used. The combination with nitrites 1) does not remove beta-blockers bradycardic effect; 2) cau-

INTERMEDIATE WORK
DOUBLE PRODUCTS (1080 kgm)



HEART RATE SYSTOLIC PRESSURE

FIG. 13

ses a slight further arterial pressure reduction; 3) removes the ventricular volume and the slight pre-ejection period increase. The further after-load reduction and the almost complete normalization of ventricular volume will obviously produce a further MVO_2 reduction.

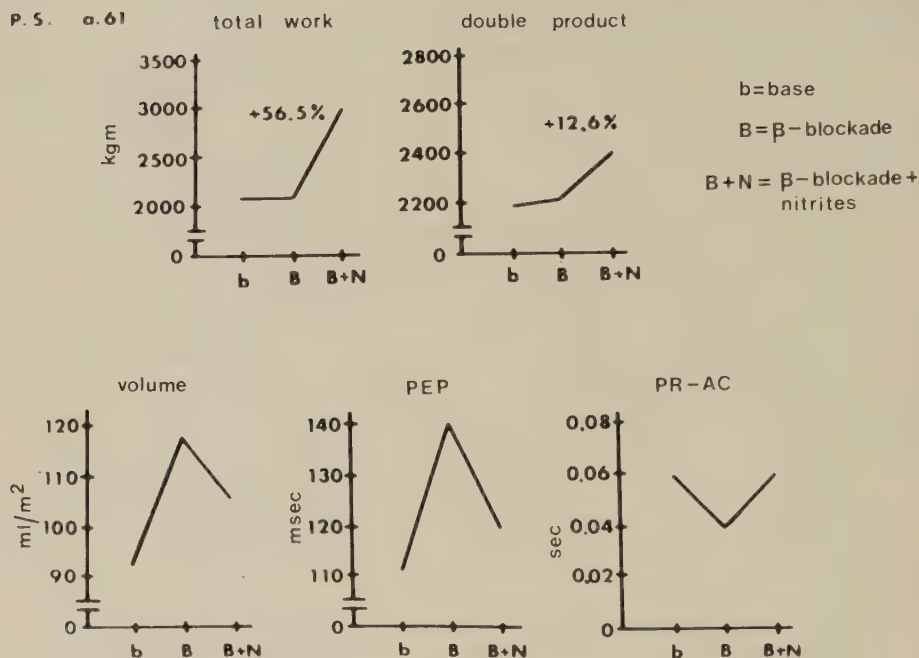


FIG. 14

Our results point out, first of all, the importance of evaluating the main parameters affecting MVO_2 ; this is the only way for a more exact evaluation of the drugs used in patients with coronary artery disease. In particular, regarding the drugs used, we must note that: 1) heart rate is not a sufficient parameter for the assessment of propranolol doses in patients with coronary artery disease, otherwise one risks reaching doses that can depress left ventricular performance; 2) one may efficiently use the so-called "anti-ischemic" propranolol property, avoiding the harmful effects on the myocardial contractility; this can be accomplished by using small beta-blockers doses combined with a complimentary drug such as nitrites; 3) with a beta-blockers + nitrites treatment one may unmask reversible dyskinesia (16) which is useful in deciding whether a myocardial revascularization operation is needed.

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Effect of biofeedback training on stress and hypertension

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Today modern man is in a constant state of mental tension which affects his health adversely, and this is an important factor in the production of hypertension. This study was undertaken to determine whether the effects of stress can be mitigated by biofeedback training and relaxation.

MATERIAL AND METHODS

Twenty patients of essential hypertension who were being treated with drugs in our clinic were selected for this study. These patients were divided into two group of 10 each. 10 patients were treated by relaxation and biofeedback training and other ten were taken as the control group.

Treatment Group

Of 10 patients in this group, there were 9 males and 1 female, the average age was 56 years, and average duration of high B.P. was 7.1 years. Original average blood pressure (blood pressure before the patients were put on any antihypertensive therapy) was 170/107 mmHg (average mean blood pressure 128 mm). With drugs average blood pressure was 158/103, mean 121 mm. At this stage these patients were given biofeedback training.

Control Group

This group consisted of 9 males and 1 female, the average age was 57 years and average duration of hypertension was 6.9 years. Average original blood pressure was 168/106 mm (average mean 127 mmHg). Blood pressure after initiation of drug therapy was 160/100 (mean 120 mm).

All patients were given the cold pressor test according to the method of Hines and Brown (1932), (1) and Master's two step exercise test. Blood pressure was recorded before and after the test. Maximum rise in systolic and diastolic

pressure during the test and the time required for it to return to the level before the test were recorded. The tests were carried out before and after biofeedback training.

Biofeedback training

The technique of biofeedback training was the same as in our earlier study. (2) It was emphasized to the patients that in order to achieve good results their voluntary active cooperation was necessary.

RESULTS

Subjective improvement was noted in most patients during the course of treatment. The patients reported that they felt better, slept better and there was improvement in their other symptoms like headache, dizziness, fatigue, chest pain, etc. Average mean blood pressure in the treated group came down from 121 to 11 mm. In addition, the drug requirement was reduced by 33%. There were no significant changes in the level of B.P. or drug requirement in the control group.

The results of cold pressor test and exercise test in both the control and treated groups before and after treatment are shown in Table 1.

TABLE 1

Results of cold pressor test and exercise test in treated group

		<i>Maximum Systolic rise BP mm</i>	<i>Systolic Recovery time in minutes</i>	<i>Maximum diastolic rise BP mm</i>	<i>Diastolic Recovery time in minutes</i>
Cold Pressor Test	Before treatment	21.2	15.8	7.3	11.7
	After treatment	12.1	9.1	3.3	5.8
	Difference	9.1	6.7	4.0	5.9
	P	<.005	<.025	<.05	<.025
Exercise Test	Before treatment	25.8	16.7	8.2	12.8
	After treatment	17.7	7.9	5.5	6
	Difference	8.1	8.8	2.7	6.8
	P	<0.025	<0.025	N.S.	<0.025

As seen in Table 1 in the treated group the maximum rise in both systolic and diastolic pressure with cold pressor test as well as exercise test was reduced. The recovery time was also reduced. Changes in the control group were not significant.

DISCUSSION

Stress plays an important part in the pathogenesis of hypertension. The resulting haemodynamic changes in essential hypertension are similar to those occurring in a normotensive person during emotional stress. (3) These changes can also be produced by direct electrical stimulation of motor centres and regions of the hypothalamus (4). It appears that repeated hypothalamic stimulation by emotional stimuli leads to sustained high blood pressure (3).

Since stress is a part of life and it cannot be completely avoided, It is important to change one's reaction to stress. The effects of stress can be modified by relaxation techniques like yoga, biofeedback training, transcendental meditation, autogenic training, zen etc.

This study showing a reduction in the rise of blood pressure and recovery time after biofeedback training indicates that the effects of stress are modified by biofeedback training and relaxation.

SUMMARY

Twenty patients of hypertension were studied, 10 were treated by relaxation and biofeedback training and the remaining 10 served as control. All patients were given the cold pressor test and Master's two step exercise test, before and 8 weeks after the biofeedback training.

Subjective improvement was noted in most patients during the course of treatment. After biofeedback training, average mean BP was reduced and the drug requirement was reduced by 33 per cent.

With cold pressor test and Master's two step exercise test, maximum rise of BP and recovery time were reduced after biofeedback training. No significant changes were noted in the control group.

This shows that biofeedback training not only reduces blood pressure but also reduces the maximum rise of BP and recovery time during stress, thus modifying the response to stress.

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Immunological factors contributing to the pathogenesis of atheromatosis

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Auto and heteroantibodies to different agents and immune-complexes were determined in the serum of control subjects of different age groups and pathological groups including diabetics, acute and chronic cardiac ischemia and family members of those patients. Up to now more than 900 diseased patients were analysed whereas the total of the different control groups correspond to 350 subjects. The C_{11} -binding test was used for the determination of immune complexes, whereas antibodies were measured by means of passive hemagglutination.

Results. Altogether, the incidence of positive tests was statistically highly significantly more frequent among the pathological groups when compared to the controls. Immune complexes are frequently present not only among diseased patients but also among their family members. Comparable results were also obtained with the analysis of milk and bovine serum albumin antibodies. The incidence was especially elevated among the family members under 20 y. of age. Anti- β -lipoprotein antibodies were present in almost 50% of the diseased patients whereas almost absent in the controls. A similar observation could be made with the determination of anti-vascular tissues antibodies. Finally, anti-tabacco antibodies were frequently encountered among cardiac patients and also among their families. This was not correlated to the habit of smoking.

Conclusion. These results suggest a strong tendency to develop autohetero-antibodies among subjects with cardio-vascular diseases. This tendency is strikingly present already among young family members of the diseased patients. A correlation was found to be present with elevated values of cholesterol, triglycerides and blood lipids. For several antibodies, a notable sex-linked difference was observed. Regardless of the pathogenic signification of these findings, which was not studied in this study, it appears that the tendency to build up auto and heteroantibodies is extremely high among children of patients with cardiac ischemia, thus preceeding the development of atheromatosis.

Nosographic, clinic and histopathologic aspects of giant-cell arteritis

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In spite of several studies, giant-cell arteritis leaves still unresolved many nosographic, clinic and histopathologic problems. From a nosographic point of view, authors don't yet agree, whether it is to be considered an independent disease (2) or it should be unified with polymyalgia rheumatica which often precedes, accompanies or follows the arteritic picture. From a clinical point of view, there are real difficulties to identify some of its visceral localizations, whether asymptomatic or oligosymptomatic. Even its most typical symptom (cephalea, in the most frequent temporal localization of the disease) does not appear well defined in its features and in its pathogenesis. Sometimes cephalaea, in fact, is completely absent in spite of the temporal artery involvement, other times it is superficial, temporal epicritic, with paroxysmic exacerbations and radiations to the nape and to the neck, and other times at last it is deep, oppressive and it especially affects the vertex and the occiput. They are obviously different manifestations of the disease and they must have equally different causes. From a histopathologic standpoint authors agree to consider it as "endo-meso-arteritis" with initial disruption of the internal elastic lamina and subsequent pleiomorphic infiltration of the adjoining strates of the media and intima, perhaps as a reaction to the initial lesion. Many authors consider the presence of the giant-cells of various types ("foreign body" or Langhans) inconstant and not essential to the histologic diagnosis. Some think the definition of "giant-cell arteritis" improper and assert that the definition of "Horton arteritis" or simply "temporal arteritis" should be preferred since it does not commit from a histopathologic point of view.

This short report refers to our personal contributions to the clearing up of the problems we have just outlined (3, 4, 5). First of all a clear-cut nosographic distinction between polymyalgia rheumatica and giant-cell arteritis seems very uncertain. In fact, practising systematic biopsies of the temporal artery in subjects affected by polymyalgia rheumatica, without clinical sign of temporal localization, Alestig and Barr (1) found out a high percentage of giant-cell ar-

teritis, many years ago. Moreover in the course of a polymyalgia rheumatica there may be silent or not silent arteritic localization, different from the temporal ones which obviously show evidence of a strict relationship or a nosographic unity of the two diseases and influence the prognosis of polymyalgia rheumatica, worsening it. Sometimes a typical polymyalgia may precede an equally typical temporal arteritis even of years; in this case the common pathogenetic background can also be proved when there is a clinical connection between the two episodes. For instance in one of our patients (4) the first classical manifestation of polymyalgia rheumatica appeared 10 years before the temporal localization and it has been followed in the time by occasional depressive episodes with the increasing of the sedimentation rate, may be in relation with the encephalic arteritic localizations oligosymptomatic and with slow evolution. Considering cepheala, we must keep separate the superficial, epicritic one, from the deep oppressive one which more often appears in the advanced phases of the disease. As we have been able to prove several times the superficial cepheala is to be related to a phlogistic involvement of the nervous terminations of the adventitia or of the periadventitial nerves. If these structures are not involved by phlogosis there is no epicritic pain. The deep cepheala, instead, which is often accompanied by diplopia and depressive symptoms should probably be related to endocranic arteritic localizations. The presence of giant-cells is a constant feature of the disease and it is particularly evident in our observation in the initial phases and it tends to decrease during the evolution towards sclerosis or after cortisone treatment. The missing finding of giant-cells may be due to insufficient observation in small and unlucky biopsies especially in the most advanced phases and in the treated cases. In our opinion before asserting the absence of giant-cells patient serial sections on a long tract of artery are necessary, also because of the frequent segmentary arrangement of the lesions. Therefore we think there are no reasons to modify the already acquired definition of "giant-cell arteritis".

In the end, in one case, in a liver biopsy, the presence of limpho-plasma-cell infiltrates, especially in the portal spaces, was observed (5). This is difficult to interpretate, but it might document the systematic character of the disease.

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Potassium during digitalization

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INTRODUCTION

It is generally accepted that digitalis glycosides inhibit the sodiumpotassium dependent ATPase in the cellular membranes (Askari 1974). An inhibition of this enzyme leads to a decrease in the active transport of sodium and potassium across the cellular wall. Thus it could result in a decreased intracellular potassium level, as demonstrated in erythrocytes by Kettlewell (1972), Aastrup (1974 a, b) and others (Loes 1978).

The present purpose was to study if there is a general decrease of the intracellular potassium during digitalization.

METHODS

Twenty persons were studied prior to and after about 40 days of digoxin intake. Ten were out-patients, 38 to 77 years of age (mean 62 years), weight 48 to 131 kg, (mean 79.7 kg). All were digitalized because of mild heart failure. Ten were healthy volunteers, 23 to 37 years of age (mean 28 years), weight 64 to 94 kg (mean 74 kg). The digoxin dose given varied between 0.13 and 0.50 mg per day, and the digitalization was performed with no extra loading dose. The degree of digitalization was followed by estimating the digoxin concentrations in serum with a modified radioimmunoassay technique and by following the change in the QS₂-index. The latter was obtained from the electrocardiogram and the phonocardiogram. The serum potassium concentration was measured by routine flame photometry. The total body potassium was measured as ⁴⁰K in a whole body counter. In nine of the patients and five of the volunteers a muscle biopsy was taken from the femoral quadriceps muscle, by technique introduced by Bergström (1962), and its potassium content was estimated by neutron activation technique.

RESULTS

After the digitalization the patients had a serum digoxin varying from 0.32 to 1.90 nmol/l, the volunteers a serum digoxin from 0.51 to 1.40 nmol/l. Six of the patients and three of the volunteers were within the therapeutic range

(1.0 to 2.4 nmol/l). The QS_2 -Index decreased in the patient group from 547 to 526 msec and in the volunteer group from 533 to 516 msec. These changes are significant ($p < 0.01$).

The serum potassium decreased from 4.4 to 4.3 nmol/l in the patients and from 4.4 to 4.2 nmol/l in the volunteers. The total body potassium decreased 7.2 per cent in the patient group ($p < 0.05$), and 8.7 per cent in the volunteer group ($p < 0.01$). The muscle biopsy potassium decreased in the patients from 45.3 to 43.3 nmol/100 g fat free solids ($p < 0.05$), and in the volunteers from 45.7 to 43.3 nmol/100 g fat free solids ($p < 0.05$), corresponding to a decrease of 4.3 and 5.4 per cent respectively.

CONCLUSION

During digitalization there is a 5-10 per cent decrease in the total body potassium and in the skeletal muscle potassium.

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Subendocardial infarction - a challenge

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INTRODUCTION

Myocardial infarction has been attributed to be associated with elevation of the ST segment and subsequent development of Q waves. Hence, myocardial infarction without ST segment elevation and Q waves has been thought to be a benign syndrome and treated accordingly. However, recent work by Madias, Rigo, Fuster and others has created significant interest in this subject. The accumulated clinical experience paints a less optimistic picture for myocardial infarcts accompanied only by depression in ST segment and T wave changes. It has been observed that not only is there a higher incidence of sudden deaths and electrical instability in these patients but there is also a large percentage of patients developing post infarction angina. But here it is heartening to note that there is much lesser incidence of pump failure for which extensive myocardial neurosis is necessary.

In a three years follow-up, Levy found an incidence of sudden death of 33% in patients with subendocardial infarcts compared to 15% in patients with transmural infarcts. These patients also had a higher incidence of angina (61% against 35%) and recurrence of infarction (26% against 12%). Taking into consideration all these factors, it is evident that subendocardial infarcts should not be considered a lesser evil, instead this should be given a very serious consideration as far as management is concerned. Coronary angiography done on patients with subendocardial infarction by Fuster suggest that there is abundant collateral circulation in patients with subendocardial infarcts which prevents neurosis but there is a large perinfarction zone of ischaemia. This is responsible for potentially lethal arrhythmias as well as angina even though the conclusions drawn from these studies could be open for discussion, there is no room for any doubt that subendocardial infarcts needs closer attention. Obviously there were attempts to study these patients under various categories, like only ST change, only T wave change and a combination of both. Doubts have been expressed about the prognostic importance of a group of patients who have the changes reverting back to normal within the next few days or weeks.

Even though a large number of studies are now available on the behaviour pattern of subendocardial infarction, exact diagnostic have not been defined. For instance, the criteria committee of the New York Heart Association in

their latest addition suggest that the ECG evidence should consist of depression of ST segment in all the leads except AVR and the depression of ST segment if fixed for several days rather than disappearing in minutes or hours as is the case with coronary insufficiency. Rigo considered serial ECGs' showing ST segment changes with or without T wave abnormalities persisting for at least 24 hours. These obvious problems in the diagnostic criteria or subendocardial infarction led Willerson to use myocardial scintigrams in diagnosis of subendocardial infarct.

In a country like ours where diagnosis of myocardial infarction is based primarily on clinical impression and very few simple elementary tests like ECG and sometimes serum enzymes are done, diagnosis of subendocardial infarction poses a challenge. In view of this, it was decided to review patients admitted to the coronary care unit with myocardial infarction.

MATERIAL AND METHODS

Certain criteria for the diagnosis of subendocardial infarction were laid down like the absence of Q waves, depression of ST segment with coving, elevation of ST segment in the cavity lead, deep symetrically inverted T waves (5 or more than 5 mm) in 4 or more leads. The total number of cases admitted to the coronary care unit over the period of 22 months was 700. Out of these, 328 cases were diagnosed as acute myocardial infarction. Out of these, 66 had subendocardial infarction according to the criteria laid down. These do not include patients who had initial pattern of subendocardial infarct and developed transmural infarct later. It was found that one in every 5 patients with myocardial infarction had a subendocardial infarct.

TABLE 1

<i>Total No. of cases</i>	<i>Males</i>	<i>Females</i>
66	45	21
Age group		
Below 35	Nil	1
35-44	8	2
45-54	14	7
55 and above	23	11
TOTAL	45	21

It is evident from the above table, that the incidence of subendocardial infarction as far as sex was concerned was about twice more common in males as compared to females, while irrespective of the sex, 45 and above is the age group where majority of the patients are involved.

TABLE 2
Risk factors

<i>No.</i>	<i>Risk factors</i>	<i>26% Cases</i>
One	Risk factors	30% cases
Two	Risk factors	21% cases
Three	Risk factors	23% cases

TABLE 3

<i>Past history</i>	<i>Cases</i>	<i>%</i>
Angina	32	48%
Previous infarct	11	17%
Nil	31	47%

TABLE 4

<i>Clinical presentation</i>	<i>Cases</i>	<i>%</i>
Classical	44	67%
Border line	22	33%
ECG. Leads		
4	12	78%
5	17	26%
6 or more	37	56%
Investigations		
ESR	38	58%
Leucocytosis	5	8%
Serum enzymes: Normal	46	70%
Elevated	10	30%
Complications. Hypotension	8	12%
Dysrhythmia	17	26%
Failure	14	21%

It is seen from Table 2, that 26% of patients were without any risk factors, while 74% of the patients had either one or more risk factors.

Table 3 shows that almost half the patients presented for the first time with these symptoms.

On an analysis of the clinical presentation, as it is shown in Table 4, it was observed that a third of the patients did not have classical symptomatology out of the 22 patients who presented with atypical symptoms, 4 cases (18%) had elevated serum enzymes, while serial ECG's showed evidence of regression of ST-T changes in 30 cases i.e., 47%. It is a point of interest that majority of our

patients had normal serum enzymes, out of which 47% (22 Patients) had no previous history of a I.H.D.

It is indeed significant that half the patients had one or more complications, the commonest being dysrhythmia. Of the 17 patients, 13 had ventricular premature beats, where majority of these required more than one drug to control them. AF was found in 2 cases while an equal number had marked bradycardia with escape beats, associated with hypotension which responded to I.V. Abiopine.

DISCUSSION

Our study indicates that the incidence of Subendocardial infarction was 20%. Clinical history and ECG evidence were to be relied upon, for the diagnosis of Subendocardial infarction. It is observed, that the characteristic changes in myocardial infarcts like enzyme rise, raised ESR, Leucocytes and Pyrexia were conspicuous by their absence in Subendocardial infarcts. It is interesting that about half the patients presented without any past history of I.H.D. The significance of this condition is highlighted by the fact that half the patients had one or more complications which required prompt treatment. The revealing studies on Subendocardial infarction has thus focussed the attention on to their sinister nature as compared to the hitherto considered benign course of this condition.

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Usefulness of carotid sinus pressure in detecting the sick sinus syndrome

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The sick sinus syndrome is a term introduced by Lown to describe certain arrhythmias following direct current cardioversion. He had noted chaotic atrial activity, changing P wave contour, and bradycardia interspersed with multiple and recurrent ectopic beats and with runs of atrial and nodal tachycardia. Ferrer expanded the definition and included the following rhythms in the sick sinus syndrome: 1) persistent severe and unexpected sinus bradycardia; 2) sinus arrests, brief or sustained, with escape atrial or AV junctional rhythm; 3) prolonged sinus arrest with failure of a subsidiary pacemaker resulting in total cardiac asystole; 4) chronic atrial fibrillation with slow ventricular responses not caused by drug therapy; 5) inability of the heart to resume sinus rhythm following electroconversion for atrial fibrillation and 6) alternating bradyarrhythmias and tachyarrhythmias.

The diagnosis of this condition depends on the recognition of the characteristic arrhythmias which are frequently transient and may not be apparent on routine electrocardiography. Thus, there is a need to accurately measure sinus node function or sinoatrial conduction in order to identify patients with this condition.

Among attempts to evaluate sinus node function, overdrive suppression has proved to be of limited value since a majority of patients with sinoatrial disease may show results within the normal range.

The indirect measurement of sinoatrial conduction time described by Strauss and his co-workers have been found to be beneficial in detecting patients with sino-atrial disease by same workers, and without merit by others.

Crook and his co-workers concluded that no current electrophysiological measurement has diagnostic value in patients with sinoatrial disease.

In the present study we have evaluated the effect of carotid sinus pressure in a group of patients with the characteristic arrhythmias of sinoatrial disease and in a control group with no known arrhythmias. We have also investigated the relationship between the carotid sinus pressure and the sinus node recovery time.

PATIENTS AND METHOD

The diagnosis of the sick sinus syndrome was made in 30 patients during the time period from June 1975 to June 1977. Cases of drug induced sinus bradycardia were excluded. The mean age of the patients was 69 years with a range of 34 to 88 years. There were 14 males and 16 females in the series. The patients with sinoatrial disease were all symptomatic and had at least 2 of the 3 characteristic arrhythmias, sinus bradycardia, sinoatrial block or supraventricular tachyarrhythmias.

The other 20 patients were investigated with electrophysiological studies because of chronic bundle branch block or dizzy episodes. None of these patients had the typical arrhythmias of the sick sinus syndrome. The mean age of the patients in this group was 64 years with a range of 48 to 82 years. There were 9 males and 11 females.

All patients had given informed consent and none had received previous sedation or antiarrhythmic drugs within 1 week of the investigation.

With the use of local anesthesia, a bipolar pacing catheter was introduced percutaneously into the right femoral vein and fluoroscopically positioned at the tricuspid valve. The position was adjusted to obtain optimal recordings of the bundle of His electrogram. A No. 6 Fr quadripolar catheter was passed by way of the right antecubital vein and positioned along the lateral wall of the right atrium. The distal pair of electrodes was used for programmed stimulation and the proximal pair of electrodes for sensing and recording spontaneous right atrial activity. Simultaneous records of the His bundle were obtained from the bipolar electrode together with the surface electrocardiographic leads I, II and III.

The PA, AH, HV and HS intervals were recorded before atrial pacing and at atrial pacing rates of 80 to 150 beats/min. In addition, the recovery time of the sinus node pacemaker, after sudden cessation of the highest pacing rate obtained, was accomplished in all patients. The pacing was continued for 2 minutes before determining the recovery time.

Prior to carotid sinus stimulation, the quadripolar catheter was advanced into the right ventricular apex. Ventricular pacing could then be instituted if the rate fell excessively. Massage of the right and then the left carotid sinus was applied for not more than 10 seconds at a time. On many occasions, the desired effect was obtained before this time period and the manipulation was stopped. The His bundle electrogram and the right atrial deflection were obtained during this maneuver.

The corrected sinus node recovery time which is the observed sinus node recovery time minus the unpaced cycle length was calculated as well as the cor-

rected carotid sinus recovery time which is the observed carotid sinus recovery time minus the unpaced cycle length.

All recordings were obtained with a DR 12 Electronics for Medicine machine at a paper speed of 100 mm per second. A student *t* test, evaluating the differences between means of two groups, was obtained in the patients with the sick sinus syndrome. In the patients without the sick sinus syndrome, a Pearson *R* correlation was utilized.

The sensitivity of the tests was calculated. This is defined as: True positives + false negatives. In addition, the specificity of the test was also calculated. This is defined as: $\frac{\text{True negatives}}{\text{False positives}} + \text{true negatives}$.

RESULTS

In the 30 patients with the sick sinus syndrome, the sinus node recovery time was greater than 1680 m/sec. in 5 patients. Dhingra and his group¹⁰ had observed in 61 normal patients that the normal sinus node recovery time was up to 1680 m sec. Thus, only 17% of this group was detected by this parameter. The carotid sinus recovery time was 2000 m/sec. or greater in 16 patients. Franke regards cardiac asystole of 2000 m/sec. or greater as a probable abnormal response. The sensitivity of this maneuver was 53%. The average sinus node recovery time was 1367 m/sec. while the average carotid sinus recovery time was 2513 m/sec. (*p* 0.001). The corrected sinus node recovery time was greater than 450 m/sec. in 8 patients. The sensitivity of the test was 27%. (The maximum sinus pause in normal person when corrected for the basic sinus rate is 450 m/sec.) The corrected carotid sinus recovery time was greater than 450 m/sec. in 22 patients. The sensitivity of the test was 73%. The average corrected sinus node recovery time was 431 m/sec. while the average carotid sinus recovery time was 1544 m/sec. (*p* 0.02).

In the 20 patients without the sick sinus syndrome the sinus node recovery time was normal in every patient. This signifies a 100% specificity. The carotid sinus recovery time was not greater than 2000 m/sec. in any patient. Thus, a 100% specificity was also calculated. The average sinus node recovery time was 997 m sec. while the average carotid sinus recovery time was 1054 m/sec. A high correlation between these results was obtained (*r* = 0.71, *p* < 0.001).

The corrected sinus node recovery time was less than 450 m/sec. in every patient with a specificity of 100%. The corrected carotid sinus recovery time was less than 450 m/sec. in 19 of 20 patients with a 95% specificity. The average corrected sinus node recovery time was 203 m/sec. while the average corrected carotid sinus recovery time was 244 m/sec. (*r* = 0.57, *p* < 0.01).

It is of interest that the corrected sinus node recovery time and the corrected carotid sinus recovery time as well as the sinus node recovery time and the carotid sinus recovery time gave remarkably similar values. Thus, the carotid sinus recovery time is a non-invasive estimate of the sinus node recovery time in patients without the sick sinus syndrome.

No complications were observed as a result of carotid sinus stimulation.

DISCUSSION

This study revealed that the sinus node recovery time as well as the corrected sinus node recovery times can only detect a small percentage of patients with the sick sinus syndrome. However, the carotid sinus recovery time and particularly the corrected carotid sinus recovery time can detect a much higher percentage of patients with sinus node disease. A sensitivity of 73% was obtained with the corrected control sinus recovery time. Thus, compression of the carotid sinus is a major provocative test for the sick sinus syndrome.

It is of interest that Mandel and his co-workers reported seven patients with the sick sinus syndrome who demonstrated, after carotid sinus massage, an abrupt sinus arrest lasting longer than 3 seconds. In four, this response was the major manifestation of the sinus node disease.

Our study also revealed that the sinus node recovery time and the carotid sinus recovery time was remarkably similar in the patients without the sick sinus syndrome. Thus, the carotid sinus recovery time is a non-invasive estimate of the sinus node recovery time in patients without the sick sinus syndrome.

Arrhythmias and carotid sinus pressure

Cohn and Lewis first reported on the electrocardiographic effects of vagal stimulation in dogs. The changes in order of their frequency were: sinoatrial slowing, atrial conduction defects with changes in amplitude, duration and morphology of the P wave, sinoatrial bradycardia with rates ranging from 30 to 50 per minute, prolongation of the PR interval, AV block, sinoatrial arrest, nodal escapes, complete asystole and ventricular escape beats. No changes were seen in the QRS complexes.

In a study of the effect of carotid sinus stimulation in 67 subjects without heart disease, Purks noted slowing of the sinus rate in 75 per cent AV conduction defects in 10 per cent, ventricular standstill in 4 per cent and atrial standstill in 3 per cent. A response was elicited in 82 per cent of those over 40 year of age, but in only 19 per cent under 40. Heidorn and McNamara also studied the electrocardiographic response to carotid sinus stimulation in 40 normal men. Interatrial block occurred in 30 instances, sinoatrial bradycardia in 14

and PR prolongation in 12. Ventricular asystole, varying from 2.0 to 5.7 seconds in duration was produced in nine subjects. Dermksian and Lamb, studying the effects of carotid sinus pressure in 50 healthy male aviators, noted that carotid sinus pressure induced some form of cardiac arrhythmia in 16 per cent and syncopal or near syncopal episodes in 42 per cent. Only half of those cadets experiencing syncopal episodes admitted to having experienced them previously.

In our present study, PR interval prolongation was frequently observed with carotid sinus stimulation. The His bundle electrogram studies demonstrated a conduction delay at the level of the AV node. Thus, the AH interval was lengthened by carotid sinus massage.

Carotid sinus syndrome

A hyperactive carotid sinus reflex is stated to be present when digital stimulation of the carotid sinus results in marked slowing of the cardiac rate or in cardiac asystole accompanied by a fall in the systemic blood pressure. In some cases only the blood pressure drops while the cardiac rhythm remains unaffected. Franke regards cardiac asystole lasting 3 seconds or more and a decrease of systolic and diastolic blood pressure of 50 mmHg or more as clearly abnormal while a slowing of the heart rate by 30 to 50%; cardiac asystole lasting 2 seconds, and a decrease of 30 mmHg in systolic blood pressure constitutes a borderline response.

The cardiovascular response on digital stimulation of the carotid sinus was divided by Weiss and Barker into three types 1) the cardioinhibitory type with bradycardia or asystole with or without systemic hypotension; 2) the vasodepressor type not associated with cardiac slowing and 3) a primary cerebral type not accompanied by either systemic hypotension or bradycardia. The cardioinhibitory type is by far the most common variety of the hyperactive carotid sinus reflex. Its incidence has been estimated to vary from 34 to 78% among persons with sinus hypersensitivity. The pure vasodepressor type is rare, making up only 5 to 10% of the cases of the hyperactive sinus reflex. The primary cerebral type is exceedingly rare and some workers question its existence.

Precipitating and predisposing factors

The carotid sinus reflex is abnormally active in the presence of cardiovascular disease. Arteriosclerosis, hypertension and coronary artery disease all predilect to sinus hypersensitivity. Digitalis and cholinergic drugs (neostigmine and acetylcholine) can increase carotid sinus sensitivity. Rudnikoff observed that administration of insulin can increase the vagal effects resulting from carotid sinus stimulation. An alteration in pH towards the acidotic side, can also increase carotid sinus sensitivity.

Decreased sensitivity of the carotid sinus mechanism occurs with fever, anemia, hyperthyroidism and other conditions accompanied by overactive sympathetic tone. Sympathomimetic drugs (isoproterenol and ephedrine) and vagolytic drugs (atropine, procaine-amide and quinidine) can also decrease the sensitivity of the reflex. Our present study reveals that in patients with the sick sinus syndrome, the carotid sinus reflex is also abnormally active. Further, this may be the main provocative test in patients with sinus node disease.

Mandel and his co-workers commented on the abnormal parasympathetic activity in patients with the sick sinus syndrome. Dighton also speculated that atrial cholinesterase activity is reduced in sinoatrial disease, thus leading to a buildup of acetylcholine in atrial tissues. With carotid sinus pressure, sinus arrest might be caused by abnormal carotid sinus sensitivity or it might be caused by either abnormal atrial sensitivity to acetylcholine or by excess local buildup of acetylcholine due to the inactivation of atrial cholinesterase.

The similarity of the carotid sinus recovery time and the sinus node recovery time in patients without the sick sinus syndrome was a striking finding. The explanation for this observation was not investigated in this study. However, the normal integrity of the sympathetic and parasympathetic nervous system is undoubtedly tested by these two maneuvers.

High dosage intravenous furosemide in refractory congestive heart failure

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Furosemide is a potent diuretic agent in wide use for treating edema due to renal, liver or heart failure.

Although high dosage furosemide (f.) therapy (1000-2000 mg/day) has been commonly used in patients with renal disease, the use of such dosages has not been reported in congestive heart failure without renal impairment. There has been hesitation in administering f. in such dosages to patients with normal renal function for fear of producing excessive dehydration resulting in hypovolemic shock.

Reports of f. administration in dosages of 2000-4000 mg/day orally to hypertensive patients with no untoward side effects prompted us to try, in a controlled study, high dosage administration of the drug to patients with severe biventricular congestive heart failure who fail to respond to f. in standard dosage, 80-200 mg/day orally or intravenously, even when given in combination with thiazides, aldosterone antagonists and digitalis. In patients suffering from severe congestive heart failure as shown by cardio-thoracic ratio > 0.48 , maximal jugular venous engorgement and pitting dependent edema to a depth of more than 5 mm over both calvs or sacrum participated in the study.

All the patients required hospitalization for severe and progressive cardiac edema despite treatment with digitalis, f. (80 to 400 mg/day), and aldosterone antagonist or moduratic prior to hospitalization.

All the patients had no significant renal impairment.

Each patient was given at least one continuous drip infusion of 1 gram f. which had been added to 400 ml normal saline at a rate of 4 mg/minute.

The mean weight loss during the 24 hour period following treatment was 4.1 ± 3.1 kg and mean urinary output was 2710 ± 1840 ml greater than on previous day.

With a single exception who showed no diuretic response whatsoever, all the patients showed an increase of 2000 ml or more in urine output during the 24 hour period following the start of the infusion.

Four of the ten patients have died from severe congestive heart failure since this therapeutic trial was started; in none of them could their death be ascribed

to the administration of high dosage f. Two of the four patients succumbed after being maintained in a satisfactory state, by means of repeated high dosage f. treatments, over a period of 2 month and 2 years respectively. No significant changes in serum sodium, BUN and acid were encountered. Potassium and Chloride decreased significantly but in none of the patients were the post f. values outside normal limits. A statistically significant but clinically insignificant hyperglycemia was caused by this therapy.

Mean blood pressure decreased significantly but in no case was any hypotensive change of consequence observed.

We did not encounter any of the commonly reported complications of high dosage f. such as deafness, hyperuricemia with precipitation of podagra, hyperglycemia or vascular collapse.

Furosemide in the usually administered dose blocks sodium reabsorption primarily in the ascending limb of Henle's loop. In higher doses it may also reduce sodium reabsorption in the proximal tubule as it has been shown by Seldin in 1966. Furthermore, f. in high doses has been shown to increase renal perfusion.

It is possible that the increased efficacy of high dosage f. administration may result from these two effects in combinations.

The present series of cases show that such a therapy is both safe and efficient. It should, however, be administered at a rate not exceeding 4 mg/min to prevent deafness and under strict surveillance of fluid and electrolyte balance.

The impressive results of this study with no side effects warrant more extensive clinical investigation.

An influence of strophantin and glucagon upon hemodynamic in myocardial infarction

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Under the study were the hemodynamic effects of an isolated and joint use of strophantin (doze 0.25 mg) and glucagon (doze 5 mg), which were inserted into a vein for one time. 77 infarct patients with hard heart failure during the acute period of their disease were under the observation.

Hemodynamic control was defined with the help of an indirect method — an integral rheography. 180 dynamic observations have been made.

The results of these observations showed that the joint use of strophantin and glucagon was accompanied by a synergic inotrop effect, the degree of which was equal to the sum of effects from the isolated application of the preparations. At the same time, the heart output increased in 19.2%, left ventricular work index — in 32.4%, its capacity — in 38.5% the average speed of increasing of the inner pressure in a ventricle — in 25.5%.

The obtained results permit to recommend the combined application of the preparations in cases of hard cardiac failure during an acute period of myocardial infarction after that when effect from their isolated application proved to be not enough.

Disorders of the magnesium metabolism and myocardial infarction

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More and more it seems suspicious that magnesium deficiency can be important for the advancement of myocardial infarction, which may be due to different causes (1, 3, 4, 7).

In 20 patients with myocardial infarction serum magnesium and calcium concentrations were measured in the first 14 days following myocardial infarction. Every case of myocardial infarction was documented by electrocardiography and increased enzyme activity. From each patient 15 blood samples were taken. The 1st sample was received within the first 24 hours after onset of acute myocardial infarction. Further samples were collected in the following 14 days.

For the determination of magnesium and calcium in the serum, the atomic absorption spectrometry has been used.

The average values of serum magnesium and calcium concentrations are graphically illustrated in Figure 1.

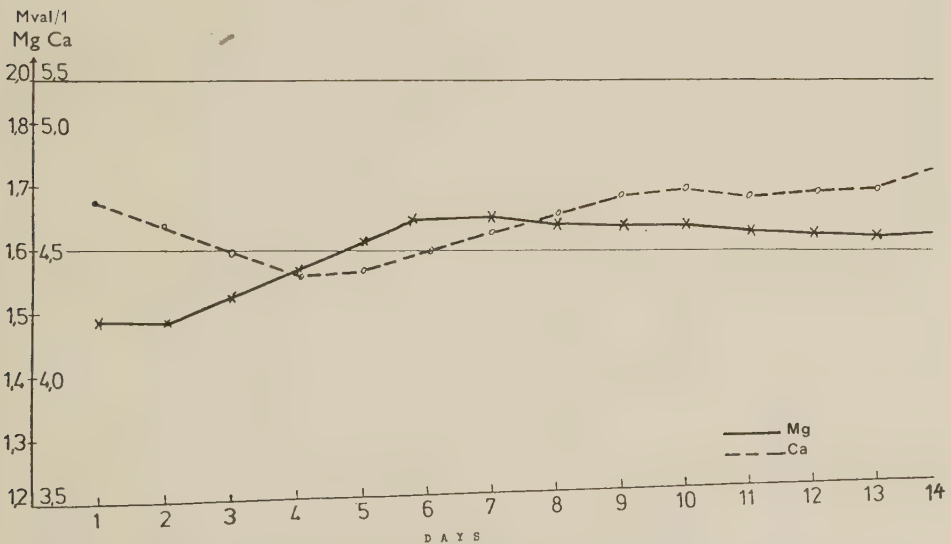


FIG. 1. Average serum magnesium and calcium values following myocardial infarction.

The lowest serum magnesium concentrations were found on the 1st and 2nd day post-infarction. Serum magnesium level raises slightly from the 3rd to the 6th day, attaining the normal range already on the 5th day. Serum calcium levels were found to be in the normal range except on the 4th and 5th day.

The results we found were in agreement with those of other investigators, who took their blood samples at the same time, that means in the first 48 hours following myocardial infarction (3, 8).

As magnesium measurements in patients have not been obtained before myocardial infarction, there is no certain proof that magnesium deficiency was already present at this time. Nevertheless, Magnesium does occur in man and may contribute cardiopathies of diverse etiology (2, 5, 6, 7, 9). For the final elucidation of this problem, it might be necessary that magnesium measurements should be regularly obtained in patients with coronary heart disease.

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Results of a national therapeutic trial conducted in 10,000 hypertensive patients by 2,000 general practitioners

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SUMMARY

1. 10,294 hypertensive patients were treated and followed by 2,200 general practitioners under the supervision of 130 cardiologists and nephrologists.
2. The treatment groups, randomly allocated, were designed to use 3 distinct antihypertensive drugs, administered alone, and combined two-by-two.
3. Some 75% of patients had a supine diastolic blood pressure of less than 95 mmHg after 4 months treatment.
4. 12% of patients have dropped out by 4 months from entry; no clear relationship was established between side effects and drop out.

INTRODUCTION

At present medical therapy of arterial hypertension usually aims at maintaining diastolic blood pressure below 95 mmHg with a minimum of side effects. The purpose of this multicentre study was to compare, on a large scale, the efficacy of and tolerance to three antihypertensive drugs: timolol maleate, methyldopa and hydrochlorothiazide 50 mg combined with amiloride hydrochloride 5 mg ("Moduretic"), administered alone and combined two-by-two [Brailowsky in press; Frithz (4), Webster (21)]. Particular attention was paid in this large scale study to patient characteristics and the existence of associated risk factors for ischaemic heart disease [Lew (13), Weinsier (22)]. No similar study has been conducted in France before; the only comparable studies known to us have been carried out abroad, for example, in Great Britain [Medical Research Council (14)].

METHODS

2,200 general practitioners, distributed throughout France, treated 10,294 male and female patients, aged between 30 and 65 years, who had sustained

hypertension as demonstrated by a supine diastolic blood pressure (5th phase) of 95 mmHg or higher, but under 130 mmHg. Patients were admitted to this study if they had never been treated, or if they were inadequately treated, or if the previous treatment was not well tolerated. Patients already treated with beta-blockers were however excluded from the study. The exclusion criteria comprised any of the known contra-indication of each of the test drugs, or any serious disease.

The treatment groups, randomly assigned, were designed to use 3 distinct primary regimens for one month (Fig. 1): timolol (TML) 10 to 20 mg daily

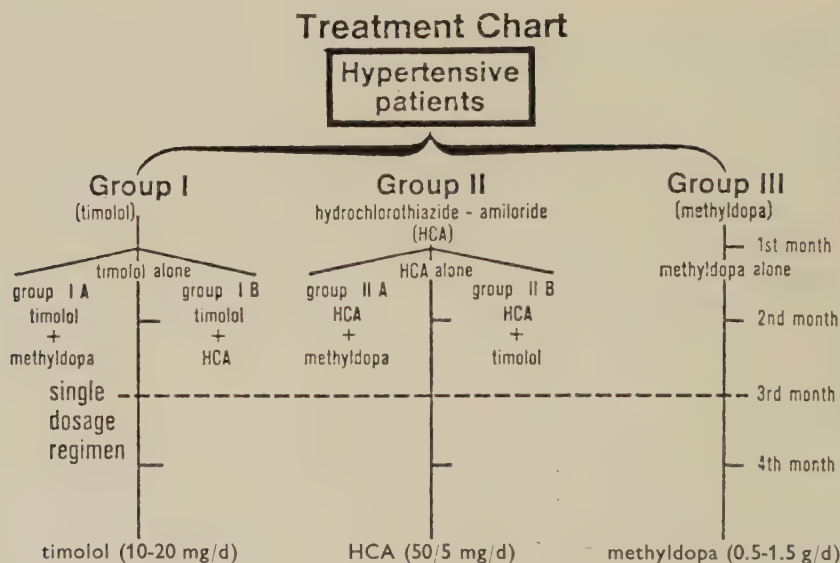


FIG. 1

(group I); hydrochlorothiazide 50 mg with amiloride 5 mg "Moduretic" (HCA) daily (group II); methyldopa (MET) 500 mg daily (group III), with supplementary drugs as follows for those responding inadequately. Methyldopa (group IA) or "Moduretic" (group IB) were the supplements for timolol; methyldopa (group IIA) or timolol (group IIB) were the supplements for "Moduretic". Group III received methyldopa alone in increasing doses up to 2,000 mg per day. Where timolol was given either alone or in combination, it was during the fourth month administered in a single dose per day [Frithz (4)]. Patients were examined monthly for four months. At each visit blood pressure was measured and side effects were recorded. The data were analysed by computer.

RESULTS

1. Epidemiological Aspects

The study included 10,294 patients, 48% males and 52% females, with an average height of 1.71m and weight of 78 kg for males and 1.60m and 68.9 kg for females. The distribution of occupational categories showed no significant difference from that of the national population except that supervisory personnel and the liberal professions were under represented. In 86% of the patients studied the known duration of hypertension was 5 years or less. 52% of the patients admitted to the study had not received previous antihypertensive therapy. 33.5% of the patients were smoking more than 15 cigarettes a day, 43.9% had cholesterolemia of more than 2.5g/l and 64.6% were more than 10% overweight as compared to the ideal weight calculated from the Lorentz formula.

2. Therapeutic results

Following one month of monotherapy, patients whose blood pressure was not normalized were divided into 5 groups: (TML + MET) — (TML + HCA) — (HCA + MET) — (HCA + TML) — (MET) alone. After one month of treatment, a certain number of patients stayed on monotherapy, others were switched to dual therapy. Therapeutic results were studied according to the 8 treatment groups: 3 groups on one drug, 5 on two drugs. When methyldopa was given at a dose of $> 500\text{mg/day}$ (IIIA), it was considered as dual therapy (see table I).

TABLE 1

Group	No.	Systolic B.P. mmHg	Diastolic B.P. mmHg	Heart Rate/Min.	Δ Systolic mmHg	Δ Diastolic mmHg	Δ Heart Rate/Min.
I	617	178.9	105.2	82	—35.3	—22.5	—13.6
II	480	178.2	103.6	78	—33.7	—20.1	— 2.8
III	642	183.3	104.5	78	—32.9	—19.4	— 3.0
IA	1175	192.7	109.7	82	—37.8	—22.2	—11.5
IB	1272	190.2	109.2	82	—37.7	—22.5	—12.0
IIA	1433	191.8	109.1	79	—38.4	—22.2	— 3.8
IIB	1403	190.1	108.6	82	—39.0	—22.2	—11.2
IIIA	781	193.3	109.7	79	—34.4	—19.7	— 3.4

a) *Efficacy.* After 4 months of treatment, for each treatment group, the number of patients, the mean supine systolic and diastolic blood pressures, mean supine pulse rate, and the mean reduction in supine blood pressure were

as shown in Table 1. Some 75% of the patients had a diastolic blood pressure less than 95 mmHg after four months of treatment (Fig. 2 and 3).

b) *Tolerance.* Approximately 25% of patients had one or several side effects during the study period.

At least 52% side effects appeared during the first month of therapy, i.e. during the period of monotherapy, whatever the treatment was. 61.6% of side effects disappeared without a change in dosage and 16.8% disappeared following simple dosage reduction. 21.6% required discontinuation of therapy (Fig. 4). Side effects were divided into the 4 main categories, i.e.: gastro-intestinal, central nervous system (CNS), including asthenia, cardiovascular (CV) and various, more pronounced for CNS in groups with methyldopa and for CV in groups with timolol.

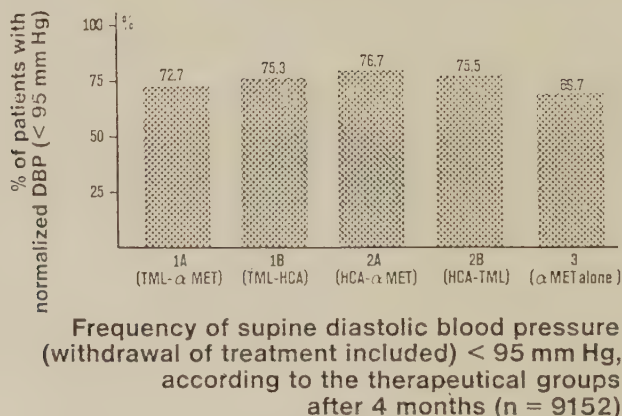


FIG. 2

Dosage schedules according to treatment groups

The most frequently used dosage in the HCA + TML groups was 1 tablet of the diuretic for one or two tablets of the beta-blocker (45% of patients in these 2 groups). In the methyldopa group, the dosage required to obtain results equivalent to the dual treatment rarely (17%) exceeded 1g/day (Fig. 5).

DISCUSSION

1. Epidemiological results

This study enabled us to collect a certain number of data concerning a population of 10,000 hypertensive patients.

MONOTHERAPIES

	SUPINE			UPRIGHT		
	TAS	TAD	Pulse	TAS	TAD	Pulse
MET —HCA	1.0 ^{NS}	0.8 ^{NS}	0.5 ^{NS}	0.1 ^{NS}	1.1 ^{NS}	2.0*
MET —TML	2.8*	5.2*	19.6*	1.5 ^{NS}	4.0*	20.7**
HCA —TML	1.8 ^{NS}	4.1*	17.8*	1.6 ^{NS}	2.7*	18.7*

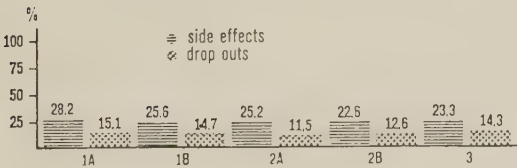
DUALTHERAPIES

	SUPINE			UPRIGHT		
	TAS	TAD	Pulse	TAS	TAD	Pulse
TML HCA + — + HCA MET	0.8 ^{NS}	0.9 ^{NS}	21.5**	0.8 ^{NS}	0.9 ^{NS}	20.4**
TML TML + — + HCA MET	0.7 ^{NS}	0.4 ^{NS}	1.0 ^{NS}	0.6 ^{NS}	0.8 ^{NS}	2.9*
TML HCA + — + HCA TML	1.4 ^{NS}	0.9 ^{NS}	2.1*	0.3 ^{NS}	1.1 ^{NS}	2.5*
TML + — α MET > MET	3.7*	4.5*	20.4**	3.6*	4.7*	6.9*
HCA + — α MET > MET	3.9*	4.3*	1.5 ^{NS}	0.7 ^{NS}	4.1*	2.2*

NS: not significant
*: significant
**: very significant

FIG. 3. Significance test.

% of patients with one or several side effects,
according to treatment groups
and % of drop outs from treatment



61.4% spontaneously disappeared
without any changing dosage regimen

FIG. 4

Obesity and hypercholesterolemia appear to be more frequent in hypertensive patients than in healthy individuals as was already shown by Tcherdakoff (19) and Hannut (7).

2. *Results of treatment*

In the group of 4,579 hypertensive patients treated with methyldopa alone or in combination blood pressure values became normal in more than 60% of the cases, with relatively small doses (50% of the patients receiving less than 1g/day). These results are better than those obtained with methyldopa in hospital studies which show an average of 45 to 50% of good results (15, 16).

The therapeutic results obtained with a combination of a beta-blocker and a diuretic were particularly conclusive. Among the 3,772 patients treated initially with either hydrochlorothiazide and amiloride or with timolol, 71% (2,675) patients needed a combined treatment of one tablet of hydrochlorothiazide and amiloride and one or two timolol tablets in order to obtain normalization of their blood pressure which occurred in 80% of the cases (if withdrawals from treatment are included).

We may conclude that the association of a beta-blocker with a diuretic represents a remarkable approach to anti-hypertensive treatment (3). The results of our study compared to others studies of this type are equivalent (14, 20), particularly the English trial performed by the Medical Research Council W.P. which shows similar results on the basis of similar treatments.

In the 4th month of treatment, the beta-blocker given alone or in combination with a diuretic was administered on a once-a-day basis without any change in efficacy. Timolol administered once-a-day maintains its full therapeutic activity as appears to be the case for some beta-blocker (4).

Therefore the data obtained justify the association of these 3 drugs in a single tablet, since they show a synergistic activity and a duration of action allowing a one or twice a day administration.

As far as safety is concerned the results of our study are similar to those of hospital studies and other cooperative studies of this type, in particular the trial using acebutolol administered alone or in combination (18).

Approximately 25% of the patients had side-effects which mostly (61%) minor in nature, and did not necessitate a change in dosage.

Only 13% of the hypertensive patients who entered the study interrupted treatment before the end of the 4th month. Several hypotheses could explain this very low percentage: either some of the participating physicians could have omitted to mention some withdrawals or a certain number of patients knowing that they participated in a clinical study may have come to their physician more

regularly, or because withdrawals from treatment perhaps become more frequent after a longer period of treatment, than was the case in our study.

This study did not reveal whether a given therapeutic regimen is more appropriate for a given type of patient. It would therefore be of interest to make a comparative analysis of the various treatment groups in function of the different parameters characterizing the hypertensive patient: age, pulse rate, initial blood pressure and risk factors.

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Evaluation of left ventricular performance in patients with ischemic heart disease compared with their myocardial imaging

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Attention was paid to the relationship between left ventricular functions and myocardial imaging with Thallium-201. 102 patients undergone coronary arteriography was first grouped into one, two and three vessel disease including 13 patients with myocardial infarction. Ejection fraction, left ventricular end-diastolic pressure, mean Vcf, left ventricular mass, left ventricular compliance and $t\text{-dp}/dt$ were secondly obtained from cardiac catheterization. In each of the classified coronary artery disease, these functional parameters were discussed comparatively. Thirdly, myocardial imaging was carried out with time from immediately to 20 minutes after injection of Thallium-201 at left anterior oblique projection utilizing Searl gamma camera. Activities were separately obtained from the regional area corresponding to left anterior descending arterial, right coronary arterial and left circumflex arterial beds respectively. These activities were compared with the entire myocardial ones indicating them as ratio. Furthermore, before activities of subendocardium and pericardium were individually presented, the preceding profile scanning was performed through the myocardium and surrounding background to determine the border of endocardium and pericardium. Left ventricular end-diastolic pressure was almost elevated only in three vessel disease and left ventricular mass in two and three vessel disease. Ejection fraction and left ventricular compliance were decreased in two and three vessel disease. The $t\text{-dp}/dt$ was slightly increased only in three vessel disease. From these correlations in which functions, the degree of vessel disease and regional myocardial imaging identified each other, it was concluded that myocardial imaging was remarkably useful for evaluation in hemodynamics of the left ventricle, in addition to the detection of segmental coronary artery disease.

Individual factors influencing the response of a beta-blocking agent alone and in combination with a diuretic in the treatment of arterial hypertension

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103 patients with uncomplicated essential hypertension were treated in a multicentre trial with timolol + placebo in a 7-week period and with timolol + Moduretic[®] (hydrochlorothiazide + amiloride) in another 7-week period. The plan of the study is given in Fig. 1.

PLAN FOR THE MULTICENTRAL TRIAL

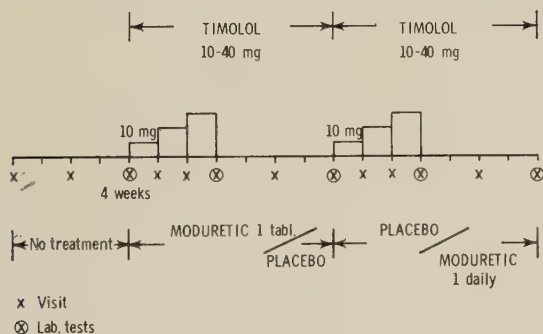


FIG. 1. Plan of the study.

Significant reductions in blood pressure (BP) were obtained on both regimens as can be seen in Fig. 2. Some of the results were previously reported (Lederballe Pedersen, 1977). The individual factors influencing the response to therapy are considered in the following.

Magnitude of the untreated BP

A significant negative correlation was found between the untreated supine mean BP and the change of this parameter ($r = -0.49$, $p < 0.001$). However, on timolol alone, the decrease in supine mean BP did not correlate significantly with the untreated BP value ($r = -0.17$, $p > 0.05$).

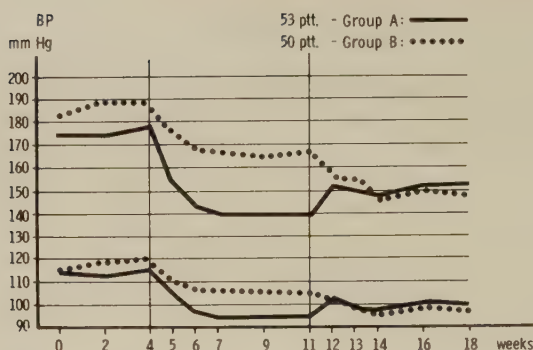


FIG. 2. Supine blood pressure (BP) during the study. Group A starting with combined therapy, group B with timolol alone.

Age of the patient

Another significant factor influencing the response in BP during the two different treatment periods was the age of the patient. As can be seen in Fig. 3, patients < 40 years were just as well regulated on timolol alone as on the combined therapy, whereas older patients generally were better regulated on the combined treatment.

The change in supine mean BP obtained on timolol alone did not correlate significantly with the age of the patient ($r = -0.07$, $p > 0.1$), whereas the change in supine mean BP caused by Moduretic showed a significant correlation with age ($r = -0.22$, $p < 0.05$). As shown in Fig. 4, the correlation between the BP reduction caused by Moduretic and age is even closer when the response in standing systolic BP is considered ($r = -0.36$, $p < 0.001$). The slope of the regression line is steeper for women than for men indicating a more marked effect of Moduretic in female patients.

Body weight

There was no significant correlation between the changes in body weight and the changes in BP during the two different treatment periods. A significant correlation was present between the weight change in the timolol period and the weight change induced by the addition of Moduretic ($r = -0.24$, $p < 0.05$) indicating that fluid retention may have occurred in some patients during timolol monotherapy.

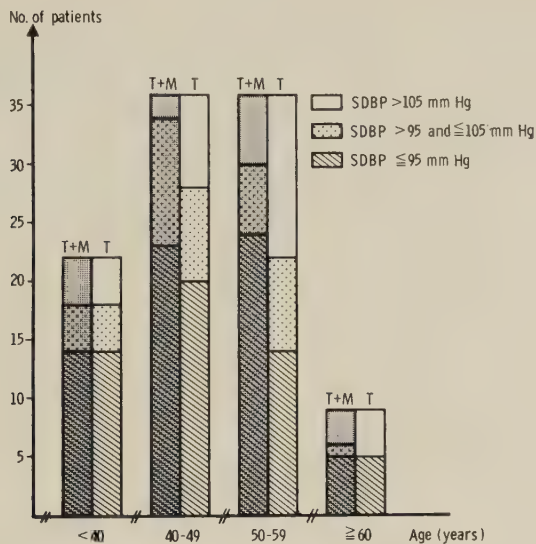


FIG. 3. Response of supine diastolic BP (SDBP) during combined therapy (T+M) and ti-molol (T) in different age groups.

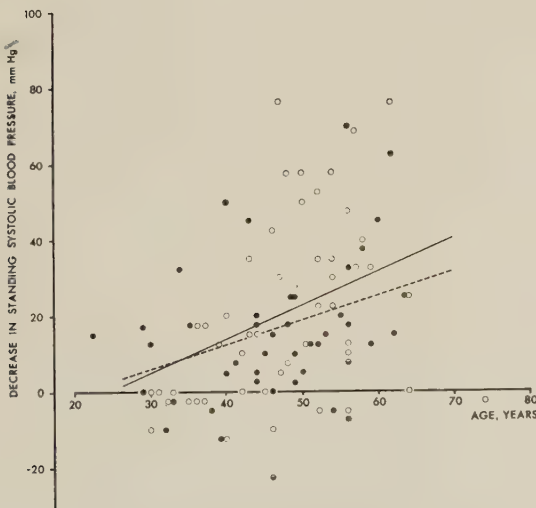


FIG. 4. Decrease in standing systolic BP caused by Moduretic as a function of age (women: ○, men: ●).

Serum potassium

During the timolol period a dose-dependent increase in serum-potassium occurred. The mean increases in the 10-, 20- and 40-mg group were 0.17, 0.22 and 0.24 mmol/l respectively ($p < 0.05$). During the combined treatment a decrease in mean potassium values was noted. The magnitude of this decrease was significantly influenced by the concomitant reduction of the timolol dose as the differences in timolol dose between the two periods correlated with mean change in serum potassium ($r = 0.86$, $n = 7$, $p < 0.05$).

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Magnesium loss in ischemic myocardial injury and infarction

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In earlier studies from this laboratory it was demonstrated that efflux of Mg from myocardial cells was one of the early obligatory accompaniments of experimental ischemic myocardial injury and necrosis and that the amount of Mg lost was, within limits, proportional to the extent of the injury sustained by the heart. In the Albino rat the depletion of myocardial Mg becomes conspicuous within 1 to 3 hours after the initiation of ischemic myocardial injury and gradually increases in extent, reaching maximum after 24 to 48 hours, when the injury is consistently at its peak.

It was reasoned that in the acutely ischemic heart, the results of such continuous efflux of myocardial Mg might, become recognizable in corresponding increases in the cumulative urinary excretion of Mg, the more so since in the Albino rat the amount of Mg appearing in the urine is fairly constant on standard laboratory chow and can be readily reduced to a stable minimum by dietary exclusion of Mg. Hence, with the elimination of any alimentary contribution to urinary excretion of the trace metal, the finding of excess urinary Mg excretion might signal the presence of acute ischemic myocardial injury and might perhaps even allow for some estimate of the extent of the damage.

The present study in Albino rats provides evidence that the early stages of experimental myocardial infarction or disseminated myocardial necrosis, which are consistently accompanied by substantial losses of Mg from the heart, are characterized by a highly significant elevation of the urinary excretion of this trace metal in the first 24 hours.

METHODOLOGY

Young adult male Albino rats of the Sprague Dawley strain, weighing 250-300 gm., were utilized. All animals were switched from standard laboratory chow to a Mg exclusive diet (Teklad Test Diets) for a total of 4 days prior to the initiation of myocardial injury. Control animals received the experimental diet with Mg added (Teklad Mg control diet). (The short period of alimentary Mg deprivation was designed to achieve both a low and stable value for urinary Mg excretion, while avoiding pronounced Mg deficiency). The animals were

kept in individual stainless steel metabolism cages and urine was collected quantitatively at predetermined intervals.

Standardized myocardial infarction was produced by ligation of the descending branch of the left coronary artery near the base of the left ventricle. Sham operations were carried out in identical fashion, including the placing of a silk ligature around the descending branch of the left coronary artery, except that the ligature was tied only loosely.

Disseminated myocardial necrosis, (non-coronarogenic), was produced by the subcutaneous injection of single dosages of Isoproterenol, in accordance with the procedure described by Rona *et al.*

At predetermined intervals (1, 3, 7, 24, 48 and 72 hours), groups of animals were killed by exsanguination in ether anesthesia. The ventricles were dissected free, weighed and prepared for determination of the total cationic content (Mg and Ca were analyzed by Atomic absorption spectrophotometry and K and Na by flame photometry). These same electrolytes were also determined in the serum obtained from samples of terminal blood drawn from the vena cava superior. The total urinary excretion of Mg was determined likewise by atomic absorption spectrophotometry.

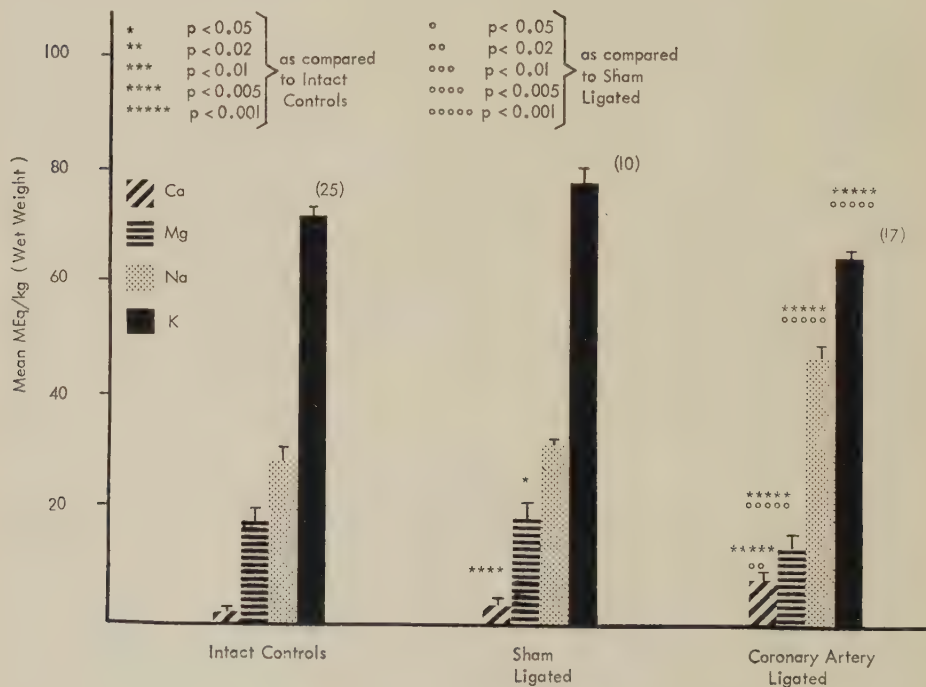


FIG. 1. Myocardial cationic content in albino rats - 24 hours post surgery.

RESULTS

The first bar graph and the following two deal with changes in the total cationic content of the myocardium produced by coronary ligation after 24, 48 and 72 hours.

It can be seen that 24 hours after ligation (Fig. 1), there is a highly significant loss of Mg and K from the heart, as well as accumulation of Ca and Na (bars on the right) if compared with normal control animals, as well as sham-ligated rats (middle group of bars). Figures in brackets refer to the number of rats in each group. It is of interest that sham ligation caused a significant loss of Mg and gain in Ca, in the first 24 hours. This is probably attributable to damage of the heart by the handling and to the injury caused by needle perforation and threading of the silk ligature.

After 48 hours (Fig. 2), sham-ligated rats (middle group of bars) can no longer be distinguished from normal control animals, whereas, in rats with coronary ligation, all four cations still show the same highly significant changes from the norm, as seen after 24 hours.

After 72 hours, when repair processes become clearly evident in the area of

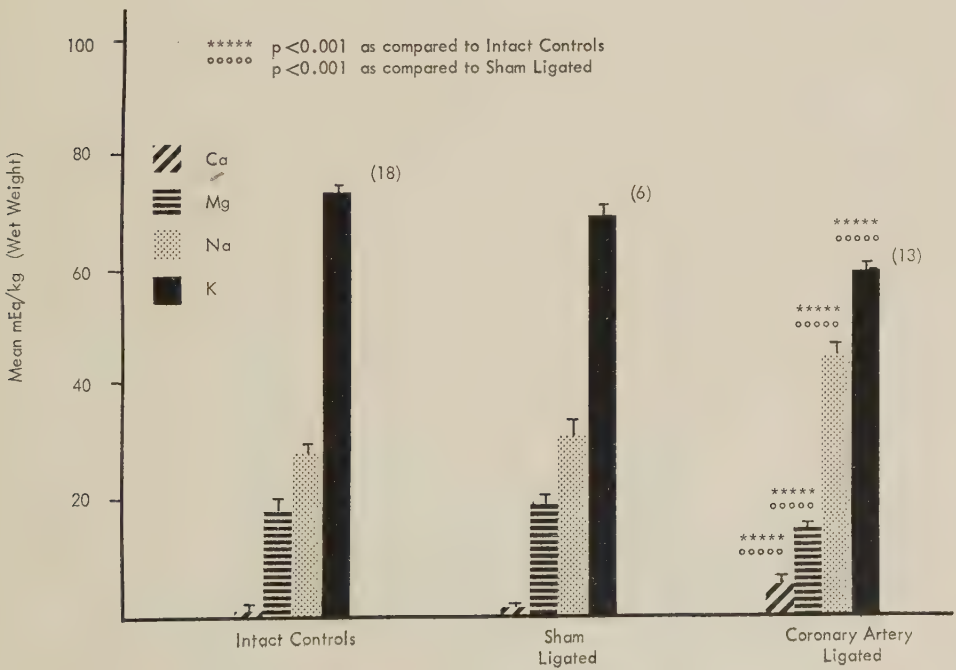


FIG. 2. Myocardial cationic content in albino rats - 48 hours post surgery.

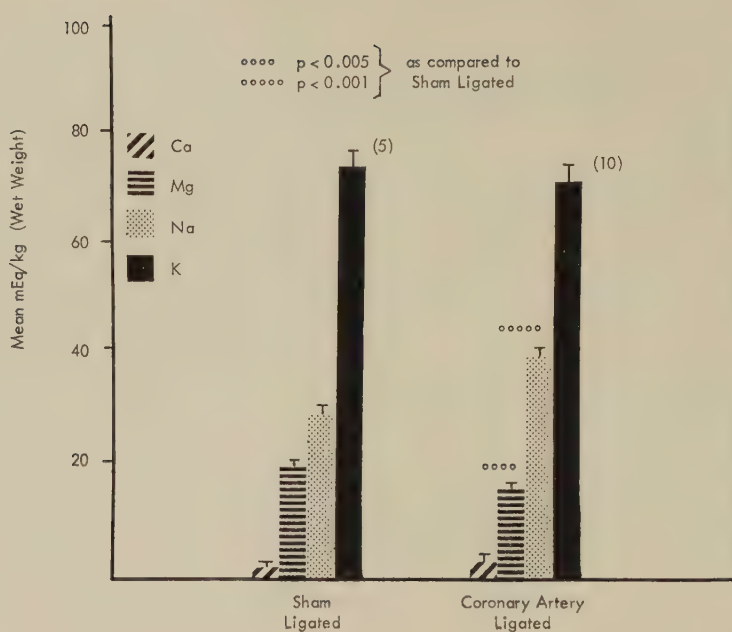


FIG. 3. Myocardial cationic content in albino rats - 72 hours post surgery.

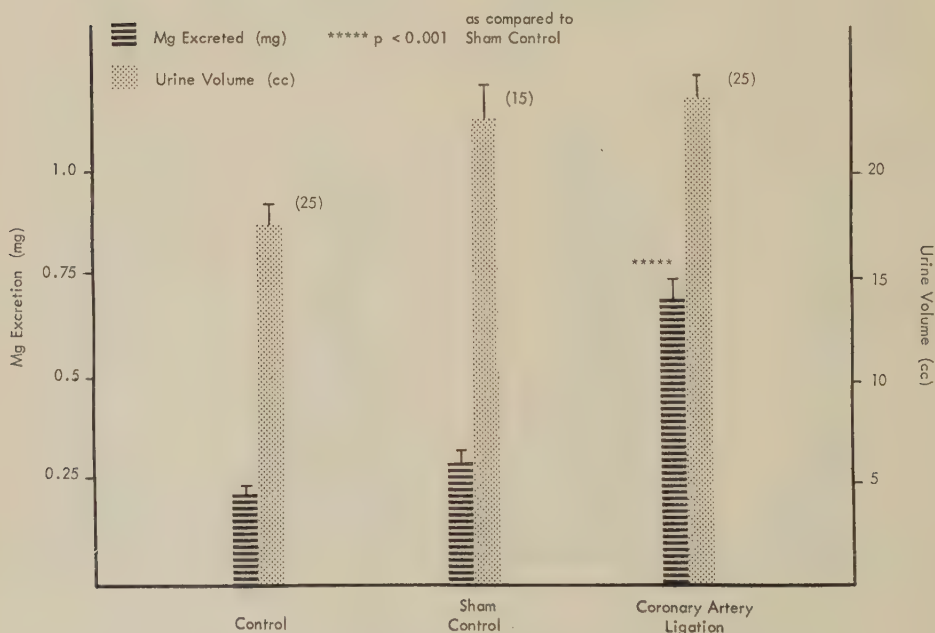


FIG. 4. 24 hour urinary magnesium excretion (Albino rats).

infarction, the values for myocardial Ca and K content are no longer significantly different from those of sham operated animals, while the deviation of Mg and Na content, though receding, still remains highly significant (Fig. 3).

The reduction of all four cationic alterations of the myocardial electrolyte content after 72 hours are particularly clearly seen, when the data from this experiment are depicted as percentage deviation from the norm and juxtaposed to the data from the 24 hour and 48 hour experiment. Increases in cationic content appear above the horizontal line and losses are depicted below the horizontal line. Note, in particular the persistence of Na gain and Mg loss after 72 hours.

The egress of Mg from heart, appears to be reflected in corresponding excess excretion of this trace metal in the urine, as seen in Fig. 4 and Fig. 6. We have as yet not excluded the possibility that ischemic tissues other than the myocardium may contribute to the Mg loss (in these two figures, the pin point bars depict 24 hour urine volume per rat and the horizontal lines bars 24 hour urinary Mg excretion).

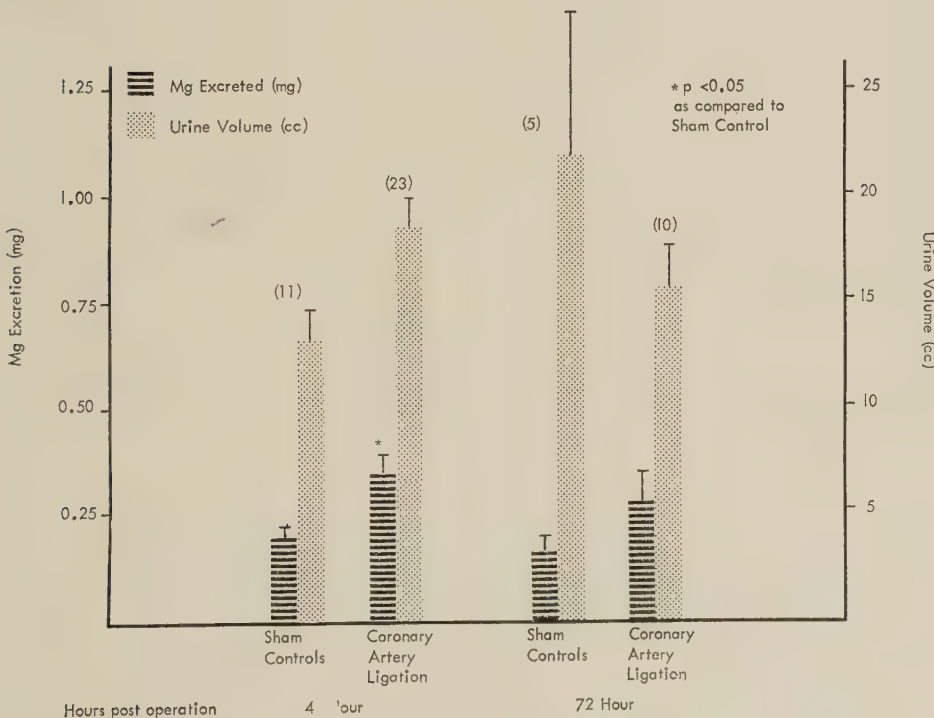


FIG. 5. Coronary artery ligation - 48 and 72 hour urinary magnesium excretion (Albino rats).

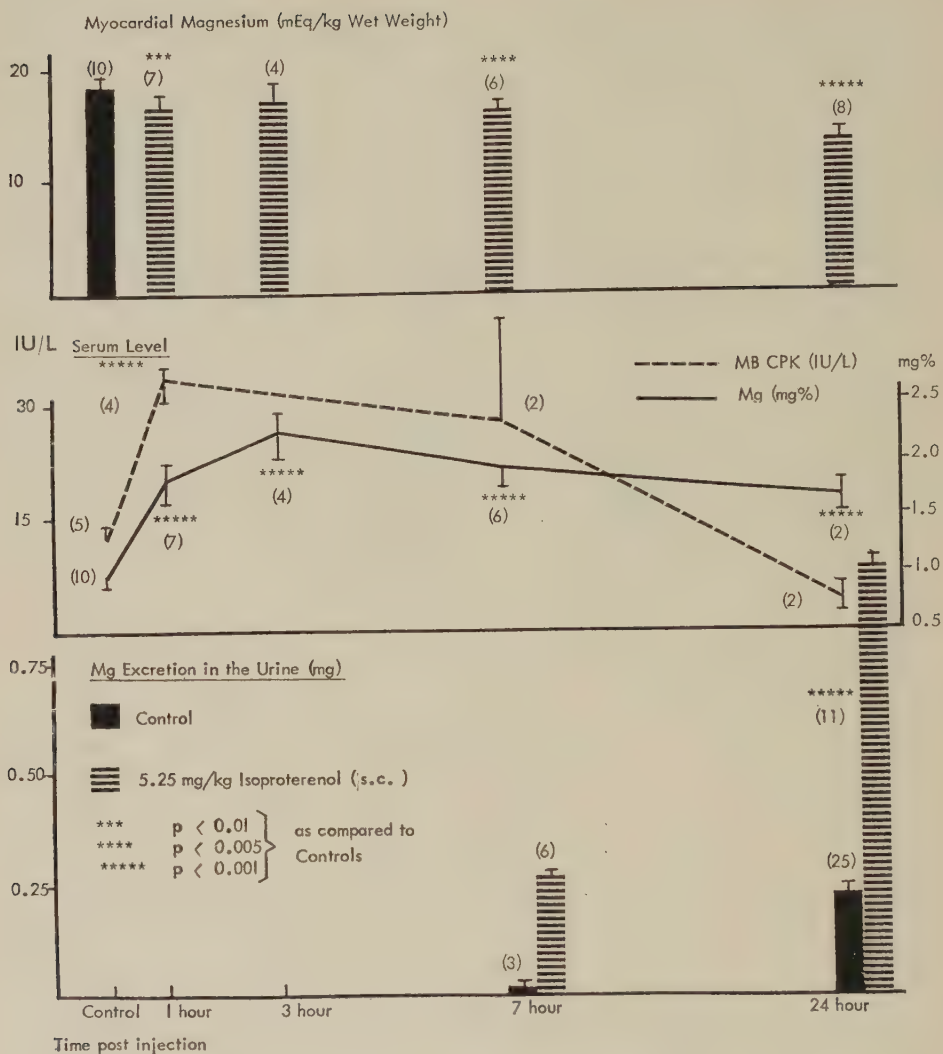


FIG. 6. Isoproterenol induced myocardial necrosis (5.25 mg/kg s.c.).

It is apparent that there is a highly significant increase in Mg excretion in the coronary ligated group in the first 24 hours, whereas in sham-ligated animals, Mg elimination remains within the normal range.

On the second day of infarction, the urinary excretion of Mg is still significantly higher than that of sham operated control animals (Fig. 5). On the third day, the difference is no longer significant. It is clear, therefore, that the bulk of the urinary loss of Mg occurs within the first 24 hours of myocardial infarction.

Time does not permit detailed presentation of our results with isoproterenol-induced disseminated myocardial necrosis but it may suffice to note that they were in line with the findings in myocardial in all aspects. As an example of the effect of a 5.25 mg/kg dose of isoproterenol within the first day—that is after 1, 3, 7 and 24 hours—revealed good correlation of myocardial Mg depletion at all intervals, with highly significant increases in serum Mg levels and cumulation of Mg in the urine in the 24 hour sample. The rise in MB CPK levels appears to parallel the rise of serum Mg levels at the 1, 3 and 7 hour intervals (Fig. 6).

In nonischemic myocardial damage (produced) by a cryosurgical probe, neither a rise in serum Mg levels nor excess urinary excretion were encountered.

SUMMARY

It has been shown that in acute ischemic myocardial injury and necrosis, produced by either coronary artery ligation or by a cardiotoxic dose of isoproterenol, Mg is lost from the heart. This loss is accompanied by a sustained rise of the Mg levels in the serum which lasts for at least 24 hours and apparently contributes to or causes corresponding increases in the urinary excretion of Mg during this time. On the second day, as the serum Mg returns to preinjury levels, the urinary excretion of Mg likewise drops into the normal range. It would appear that the extent of excess Mg excretion in the acute phase of myocardial injury (first 24 hours) might within limits, be proportional to the degree of damage sustained by the heart. More studies are needed to confirm these preliminary findings. The possibility of using the urinary excretion of Mg as a measure of the effects of therapeutic and deleterious interventions in acute ischemic myocardial injury, is at present under study in our laboratory.

3,5,3'-Triiodothyronine, 3,3',5'-Triiodothyronine and Thyroxine in acute myocardial infarction

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Changes in thyroid hormone levels during acute myocardial infarction (AMI) have been recently investigated, but disaccordance on their entity and duration still exists (1, 2). We have monitored the serum levels of thyroxine (T₄), 3, 5, 3'—triiodothyronine (T₃) and 3, 3', 5'—triiodothyronine (rT₃) over the first nine days of disease in 27 patients (pts) with AMI, using a radioimmunoassay method (3). The statistical analysis of the data was compared with normal values previously obtained in a group of 60 healthy adult volunteers (3). Considering the total cases, the difference in percentage between the mean hormone levels of the normal subjects and those with AMI (Δ %) was, on day 1, —33% for T₃ ($P < .01$), + 78% for rT₃ ($P < .01$), and —13% for T₄ ($P < .05$). Over the nine days studied the serum values of T₃ decreased and tended to stabilize themselves, at about day 9, at levels inferior to the initial values; the rT₃ returned to the normality by day 9 and it was not possible to demonstrate a statistical significance of the peak value of day 2, when compared to the value of day 1. The T₄ presented no statistically variation in time. A direct comparison between deceased and survived pts demonstrated with high significance that for T₃ the decrease below the normal levels was about twice in the deceased group, and the increase in rT₃ was about four times greater. No difference was observed for T₄. The behaviour of rT₃ of the survivors during the nine days was essentially stationary with a return to normal values on day 8. In the deceased group there was a significative peak on days 2 and 3 with successive gradual decreases, resulting still above the normal values on day 9. Subdividing the total cases in three therapy groups, it appeared that the levels of T₃ and T₄ on day 1 were not significantly different. The increase in rT₃ on day 1, comparable in the two groups receiving corticosteroids and digitalis respectively, was about 50% greater than the standard therapy group. With respect to time, the behaviour of T₃ did not demonstrated significative differences both in Δ % and upon variance analy-

sis. Variance analysis did not demonstrate significative differences of T4 among the therapy groups, although the corticosteroid group presented a significative decrease below the normal values in days 3 and 4. The behaviour of rT3 revealed a significative peak on day 2, double the value of day 1, both in the digitalis and corticosteroid groups, with a tendency to return to the normality by day 9. The peak in the standard therapy group was not statistically significative and returned to the normal levels by day 4. Variance analysis demonstrated the significance of these differences. We found that the severity of the disease, the diet, the therapy with corticosteroids and with digitalis must be considered significative determinants of the altered thyroid hormone levels in AMI.

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Community control of hypertension programme in Cuba

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The Programme is using the criteria and methodology recommended by the World Health Organization (WHO) (1) and the Pan American Health Organization (PAHO). It is sponsored by WHO.

It works into the framework of the Policlinic, the basic health unit of the areas, and is included in the Comprehensive Health Care Programme for Adults, which represents one of the major issues in our health care system.

Aims of the Programme are: to find out all hypertensives in a community, to reduce the blood pressure to normal level by medication, then to follow-up the hypertensives with an appointment every 3 or 4 months, at the end of five years demonstrate a decrease in morbidity and mortality from hypertension. A reference area must be chosen for comparison with the study area. The Programme started on February 1974 and is being spread cautiously through the whole country. We expect it will be working in all our health areas five years from now.

A person is considered hypertensive when in three casual blood pressure measurements is at or over (2):

15 to 19 years	20 to 29 years	30 to 64 years	65 or over
140/90	150/90	160/95	170/95

Sitting position. Right arm. Diastolic at phase 5.

The situation before the Programme is implemented in a given area is as follows:

In the population 15 years or over, prevalence of hypertension in urban areas is 15%, in the rural areas 10%. Aware of being hypertensive 35%; unaware 65%. Hypertensives under treatment 40%; under control by treatment only 15% of the total hypertensives. Stage I (without LVH), 72%. Stage II (with LVH), 11%; Stage III (with additional organ involvement), 10%.

Mortality rate from stroke, 10 per 10,000 inhabitants per year.

Situation after four years with the Programme:

Blood pressure control:

Stage I: good in 54.2%; medium in 28.5%; poor in 16%.

Stage II: good in 45.2% medium in 25.5%; poor in 29.2%.

Stage III: good in 40.2%; medium in 36.4%; poor in 23.1%.

Missed appointments 30%. Dropouts 18%. A letter is sent to the missed appointments and dropouts, it yields a response rate of 50%. If no response to the letter a visit ensues to complete an interview form about the problem of the compliance and non-compliance, this yields a response rate of 68%.

To reduce the number of missed appointments and dropouts we have the collaboration of the People's Mass Organizations: Neighbour Committees, (Committees for the Defence of the Revolution; CDR), and the Cuban Women's Federation; FMC.

Mortality rate from stroke is 7 per 10,000 inhabitants per year in the areas where the Programme is operating.

Evaluation: At the beginning of the Programme a random sample was chosen both in the study and reference community for screening. In the final evaluation a similar sample will be chosen for a new screening in the two areas. Morbidity and mortality from hypertension will be compared in both areas. The cohort analysis of the hypertensives that were found at the first screening is included in the evaluation too.

Population and health workers education is a main issue of the Programme.

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Pathogenetic aspect of myocardial infarction

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The functional state of the sympathico-adrenal, renin-angiotensin-aldosterone, kallikrein-kinin systems, and the content of prostaglandins (PG) A, E, F₁, F₂ in the venous blood (radioimmunologic method) were studied parallelly with determination of hemodynamics (method of stain dilution) in 795 patients with myocardial infarction.

Characteristic of the acute period of myocardial infarction was activation of the sympathico-adrenal system, rise of the blood-plasma renin level, increase of the diurnal cortisol, cortisone tetrahydrocortisone, tetrahydro-11-desoxycortisol excretion.

Specific of the acute phase of myocardial infarction is marked activation of all components of the kallikrein-kinin system. Pronounced rise of kallikrein activity frankly correlated with fall of the blood-plasma bradykinogen, being an evidence of activation of kinin formation on the whole. This finds its confirmation in the elevated level of free kinins in the peripheral venous blood (biological method) ranging up to $27,86 \pm 3,26$ ng/ml.

Noteworthy of the first days of the acute phase of myocardial infarction was a sharp rise of the peripheral venous blood PGE level, drop of PGA and PGF₁ content. On the contrary, the PGF₂ level markedly increased, 10 times exceeding the norm.

Analysis of the reported facts permits the conclusion that the hemodynamic disorders and myocardial ischemia in focal-necrotic lesions of the heart being cardiogenic in origin, result in inclusion of a range of compensatory neuro-humoral systems. Called to compensate disorders of the hemodynamics, at a definite level these systems outpass the limits of hemostatic influences, and are transformed into factors of pathology by exerting an unfavourable effect on the metabolism and energetics of the heart.

Electrolytic modifications in refractory cardiac failure and in overdosage of digitalis

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To emphasize electrolytic disturbances in cardiac failure, and the extent to which digitalo-diuretic treatment, the deterioration of hemodynamic disturbances and overdosage of digitalis influence the variations of electrolytemia, we established the levels of Na, K, and Mg electrolytes in the plasma and erythrocytes and those of Ca and Cl in the plasma.

Five groups were observed, as follows: A) 34 healthy subjects, as the control-group. B) 38 persons with a recent onset of cardiac failure, who had not previously undergone a treatment of tonicardiac and/or diuretic. C) 81 patients with chronic cardiac failure, undergoing digitalo-diuretic treatment. D) 32 cases with "refractory" cardiac failure. E) 19 cases with cardiac failure and sign of overdosage of digitalis.

As against the control-group, the following modifications were obtained:

1. Plasmatic Na^+ decreases significantly in refractory cardiac failure and increases insignificantly in the other groups. Erythrocytic Na^+ increases highly significantly in all the groups, except in that of refractory cardiac failure.

2. Plasmatic K^+ increases insignificantly in all the groups, except in that of the overdosage of digitalis. Erythrocytic K^+ increases evidently significantly in all the groups, except in that of the overdosage of digitalis.

3. Mg^{++} displays insignificant modifications both in the plasma and erythrocytes in all the groups, except in that of recent onset of cardiac failure.

4. Plasmatic Ca^{++} displays variable modifications thus it increases slightly significantly in recent onset of cardiac failure, and decreases slightly significantly in refractory cardiac failure.

5. Plasmatic Cl^- decreases significantly in the 4 groups observed.

Effects of digoxin, potassium, and noradrenaline on isolated human pulmonary arteries and veins

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Studies on the effects of cardiac glycosides on pulmonary hemodynamics in man have given contradictory results (Akbarian *et al.* 1968, Murphy *et al.* 1971). However, a small but definite pulmonary vasoconstrictor effect has been demonstrated (Akbarian *et al.* 1968). Previous investigations on isolated human mesenteric and peripheral vessels showed a direct contractant effect of digoxin (Mikkelsen *et al.* 1978, a, b). In the present study, the effects of digoxin on isolated human pulmonary vessels and their dependence on extracellular calcium were investigated. The action of the glycoside on the responses to noradrenaline and potassium was also studied.

Specimens of macroscopically normal human pulmonary arteries and veins were dissected to ring preparations immediately after operation. The preparations were mounted in organ baths containing aerated Krebs solution (37°C), and isometric tension was recorded. In contrast to the arteries, most of the vein preparations exhibited spontaneous contractile activity. In both arteries and veins, the contractile response to potassium (K^+ , 127 mM) always exceeded that produced by noradrenaline (NA) in supramaximum concentration ($1.8 \times 10^{-5}M$). In the arteries, the NA-induced contracture was $33 \pm 8\%$ ($n = 8$) (Mean \pm SEM) of the K^+ contracture, and in the veins the corresponding figure was $20 \pm 5\%$ ($n = 7$).

In the veins, digoxin (10^{-7} – $10^{-6} M$) induced a contracture, starting immediately after addition of the drug to the organ bath and reaching a maximum within 30 min. The amplitude of the contracture was $32 \pm 5\%$ ($n = 5$) of the potassium induced response. In the arteries, digoxin ($10^{-6}M$) had no immediate effects, but caused a slowly developing contracture which after approx. 2 h. reached a maximum of $90 \pm 4\%$ ($n = 5$) of that induced by potassium (fig. 1). Digoxin ($10^{-6}M$) potentiated the NA and K^+ contractures in both arteries and veins. In the veins the response to NA increased to $211 \pm 7\%$ ($n = 5$), and the K^+ contracture to $145 \pm 5\%$ ($n = 5$) of control, respectively. In the arteries,

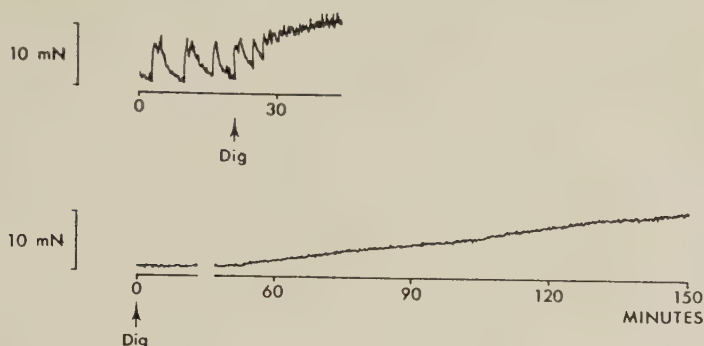


FIG. 1. Effect of digoxin (Dig), 10^{-6} M, on an isolated pulmonary vein (upper tracings), and artery (lower tracings).

the corresponding values were $169 \pm 14\%$ ($n = 5$) and $163 \pm 9\%$ ($n = 5$) of control. In both arteries and veins, the digoxin induced contractures were stable for hours, and the preparations did not relax after washing out the drug from the organ bath.

In both types of vessels the calcium antagonist, nifedipine (2.9×10^{-6} M), which completely relaxed the K^+ induced response, only had a slight relaxing effect on the digoxin contracture.

It is concluded that digoxin causes an increase in tension and potentiates the maximum responses to NA and K^+ in pulmonary arteries and veins. The digoxin induced contracture was not significantly affected by the calcium influx inhibitor, nifedipine.

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Non-invasive methods in diagnosing renovascular hypertension

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All hypertensives in our Armed Forces, especially the young, are investigated to exclude renovascular pathology, since this is the commonest cause of secondary hypertension.

MATERIAL

A total of 400 young hypertensives have been investigated over the past seven years. Of these, 40 (10 per cent) proved to have renovascular pathology, on the basis of renal angiography.

Diffuse aortitis was noted in 17 of these patients, suggesting generalised arterial disease in this group.

While 7 patients showed bilateral renal artery involvement, 4 had segmental lesions.

METHODS

Our non-invasive methods, apart from the conventional rapid sequence excretory urogram, isotope renogram and renal scan, comprised of serial Radiohippuran renal imaging and bio-electrical renal impedance.

RESULTS

TABLE 1

Proved renovascular pathology ... 40 (Total patients studied: 400)

	Positive	Negative	False positive
Excretory Urogram	30	10 (25 per cent)	18 per cent
Technetium scan	29	11 (27 per cent)	15 per cent
Isotope Renogram	32	8 (20 per cent)	9 per cent
Serial Hippuran	36	4 (10 per cent)	6 per cent

Incidence of false negative was 25 per cent in rapid sequence excretory urogram, 27 per cent in Technetium (Tc GHA) renal scan and 20 per cent in 131 I Hippuran renogram.

In view of the high incidence of false negative results with conventional non-invasive methods, we were prompted to evaluate a more dynamic isotopic method, namely, serial radio-hippuran renal imaging. This has given us only 10 per cent false negative result; thus this investigation has proved to be more reliable than other conventional screening methods.

Collectively, the isotopic methods have not missed any case of renal ischaemia.

TABLE 2

Bio-electrical renal impedance study

Total patients studied by this method	Proved renovascular (by renal angiogram)	Impedance study	
		Positive	Negative
216	22	17	5

Bio-electrical impedance study has been done as a pilot study in 216 of these patients. Out of 22 proved cases of renovascular hypertension, 5 were negative by this method. False positive occurred in 15 per cent. This method merits further study.

CONCLUSION

Our experience with non-invasive screening methods in 400 young hypertensive patients has been reported.

Rapid sequence excretory urogram, isotope renogram and Technetium renal scan have been found useful with around 75 per cent reliability.

Serial radio-hippuran renal imaging has given improved results and has been reliable in 90 per cent of cases.

Bio-electrical impedance study, a truly non-invasive and simple method, has given encouraging results but needs further standardisation and evaluation.

The above non-invasive methods, collectively, have been 100 per cent reliable as screening tests to detect renovascular pathology.

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Microvascular permeability of albumin in untreated and treated essential hypertension and during acute induced hypertension

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Transcapillary escape rate of albumin, TER, (fraction of intravascular mass of albumin that passes to the extravascular space per unit time) was determined from the initial disappearance of intravenously injected ^{125}I and ^{131}I -labelled human serum albumin in 10 normotensive subjects (BP 111/75 mm Hg) and in 18 patients with untreated benign essential hypertension (BP 177/119 mm Hg). TER was found significantly increased in the hypertensive group, average 7.6 (SD 1.1)%/hour compared with the normotensive group, mean 5.6 (SD 1.1)%/hour ($p < 0.001$). A significant positive correlation between TER and blood pressure was demonstrated in the hypertensive group, $r > 0.6$, $p < 0.001$.

Ten of the 18 patients were reinvestigated after 3 to 4 months of beta-adrenergic blockade. The treatment caused a significant reduction in blood pressure (mean 171/116 - 144/98 mm Hg) and in TER of albumin from 7.1 to 5.7%/hour, $p < 0.005$. The decrease in TER correlated significantly with the reduction in blood pressure, $r > 0.7$, $p < 0.01$.

TER of albumin was also studied in 6 normotensive subjects before and during acute angiotensin II induced hypertension (mean BP increase 42%). The TER of albumin increased significantly during the hypertensive period, average 7.2 (SD 1.1)%/hour compared with the values obtained in the normotensive state, mean 4.7 (SD 0.8)%/hour, $p < 0.001$.

The present findings support the hypothesis that hypertensive microangiopathy is caused by an increased extravasation of plasma proteins with subsequent deposition in the microvascular wall—i.e. the concept of plasmatic vasculosis.

Interpretation of the first anginal pectoris attack

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The school of cardiology of the "Coltea" Hospital represented by B. Theodorescu and his co-workers brought a great contribution to the clinical aspects of the "impeding myocardial infarction" with some systematic works from 1947 and further on.

A more particular aspect is the first anginal attack (AA) occurred to a patient who was apparently healthy, which in some cases can announce a myocardial infarction.

In a more recent study with a prospective character the evolution of the first AA was followed on a lot of 49 patients (39 men and 11 women) between 47 and 78 years old during three months.

The first group was composed of 15 patients to whom the first AA was different from the usual AA, the pain being more intense, longer, appearing after an effort or in rest. Among these, 8 patients (Pts) (53.3%) had an acute myocardial infarction (AMI) between 2 and 12 days from the beginning. All of them had at least 2 major risk factors and e.c.g. changes in rest.

The second group was composed of 35 Pts. to whom the first AA was common (the classic form described by Heberden). Among these, 10 (29.4%) had AMI between 16 hours and 31 days from the beginning.

The evolution towards AMI was as follows: either the repeating of AA more frequent and intense, or the repeating of AA same as at the beginning or stillness after the first AA, the second being correspondent with the AMI. Seven Pts. had e.c.g. changes, and 3 had normal e.c.g. All had a combination of more risk factors than those who hadn't got AMI.

Most of them who had an evolution towards AMI had not observed the treatment indicated.

As a conclusion, after our experience, an initial AA, even if it is the common form, has to be considered, until the contrary is proved, as an impending infarction, an adequate treatment being necessary at least for two weeks.

Mechanism of exaggerated natriuresis (EN) in hypertension: dose-related impairment in distal NaCl reabsorption after intravenous infusion of hypertonic NaCl solutions

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Although enhanced Na excretion after a salt load ("EN") in hypertension has been described long ago, its mechanism(s) are still disputed. In the present study graded NaCl infusions (1-1½-2 L 3% NaCl) were administered to various groups of 15 healthy subjects and 12 "normal renin" essential hypertensive patients during dehydration and under DDAVP effect. Free water reabsorption ($T^c_{H_2O}$) and fractional solute (Cosm), Na, Cl, K, Ca, Mg and P excretions were determined. The baseline relationship between Cosm and $T^c_{H_2O}$ established during high NaCl intake was significantly influenced ($p < 0.05$) by a *moderate NaCl load* only in the hypertensive patients ($T^c_{H_2O} = 0.63 \text{ Cosm} - 0.03$; $r = 0.99$ vs $T^c_{H_2O} = 0.36 \text{ Cosm} + 0.79$; $r = 0.82$) but not in the healthy subjects. On the other hand, *high NaCl loads* induced depression of the Cosm- $T^c_{H_2O}$ relationship in a dose-related fashion in *both* groups: $T^c_{H_2O} = 0.26 \text{ Cosm} + 1.09$; $r = 0.56$ (healthy); $T^c_{H_2O} = 0.27 \text{ Cosm} + 0.59$; $r = 0.82$ (hypertensive). No differences were found in phosphaturia supporting that the observed differences in $T^c_{v_{20}}$ were not due to changes in the proximal nephron. It was concluded that: 1) partly different (proximal or distal + proximal) mechanisms are used to excrete a "moderate" acute NaCl load in healthy and hypertensive persons, 2) impaired NaCl reabsorption in Henle's loop may occur after high NaCl loads also in healthy man, 3) "EN" is the consequence of a normal renal response (impaired Na transport in Henle's loop) to a certain degree of volume expansion resettled to a lower level in the hypertensive patients.

Relevance of systolic time intervals in the short-term prognosis of acute myocardial infarction

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BACKGROUND

The study of systolic time intervals (STI) in acute myocardial infarction (AMI) has yielded fairly concordant results in regard to ejection intervals (LVET), these being generally shortened; quite discordant, however, in regard to pre-ejection intervals (QS₁, ICT, PEP), whose behavior is variable. Be that as it may, the fact remains that past studies of STI have been conducted with a main view to their hemodynamic significance and little attention to their possible prognostic value; this is especially true of ICT.

METHODS AND PATIENTS

In 29 cases of AMI we recorded STI at regular intervals from the first few hours of the acute episode (Table 1) to the fourteenth day, measurements being

TABLE 1
Mean values of systolic times in 29 cases of acute myocardial infarction (AMI) during the first day of illness

		QS ₁ I _m	QS ₁ I _t	ICT	PEP I	LVETI	S ₁ S ₂ I	QS ₂ I	PEP/LVET _m	PEP/LV
Acute myocardial infarction (29 cases)	X ± s _m	104.2 ± 3.5	78.6 ± 4.15	32.5 ± 3	162.1 ± 4.7	388 ± 4	451.5 ± 5	550 ± 7.2	0.432 ± 0.01	0.360 ± 0.02
Controls (29 cases)	X ± s _m	99.3 ± 0.7	77.2 ± 0.7	39 ± 1.4	159.8 ± 3.1	404.6 ± 1.3	473.6 ± 1.4	564.4 ± 2.7	0.359 ± 0.008	0.324 ± 0.009
Significance	p	N.S.	N.S.	N.S.	N.S.	<0.01	<0.01	N.S.	<0.01	N.S.

obtained by the technic of Wissler. The patients were 23 men and 6 women, the respective mean ages being 64.8 ± 2.6 and 73 ± 3.4 years. According to the NYHA classification criteria, patient distribution was as follows:

Class I: 15 cases; Class II: 6 cases; Class III: 3 cases; Class IV: 5 cases. To expedite statistical analysis we regrouped our patients as follows:

Group A₁: patients of classes I and II

Group A₂: patients of classes III and IV.

RESULTS AND DISCUSSION

Seven patients died (overall mortality 24.1%), namely 2 of Class I and 5 of Class IV. Since our purpose was to assess the prognostic value of STI in the early phase of AMI, we devoted special attention to the behavior of these parameters on the first day. While QS₁, PEP, LVET, and the PEP/LVET ratio appeared irrelevant to prognosis, ICT was shortened to a highly significant degree precisely in patients who would die shortly or develop major electrical and/or hemodynamic complications. If we compare the cases showing a short ICT in the first 24 hours of AMI with those showing a normal or slightly increased ICT (Table 2), we can see that in our clinical material a short ICT on the first day was of definitely poor prognostic significance ("sign of the short ICT").

TABLE 2

Distribution of mortality and of ECG and/or hemodynamic complications according to the behavior of ICT

	<i>Number of cases</i>	<i>Cases with major ECG and/or hemo- dynamic complications</i>	<i>Uncomplicated cases with favorable outcome</i>
<i>Short ICT</i> ($< 39 \pm 1.4$)	17	10 = 58.82% (mortality = 7 cases – 41.18%)	7 = 41.18%
<i>Normal or long ICT</i> ($\geq 39 \pm 1.4$)	12	1 = 8.3% (mortality = 0 cases)	11 = 91.67%

SUMMARY

In 29 cases of acute myocardial infarction the authors measured systolic time intervals at regular intervals starting in the first few hours of the acute episode and continuing up to the 14th day. They found that in the first 24 hours of AMI there was a highly significant reduction of the isometric contraction time (ICT) in the higher-risk patients, and concluded that this finding might have considerable prognostic value.

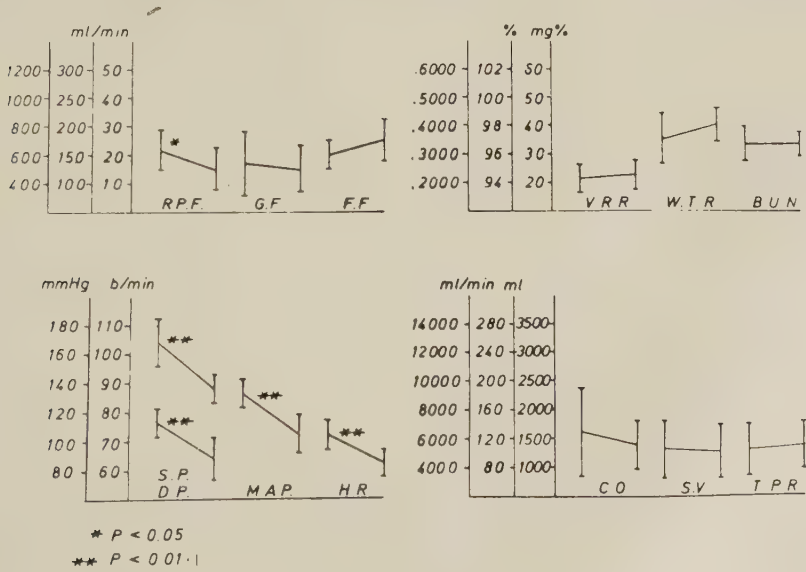
A comparative study of the systemic haemodynamics and of the renal function in subjects with essential hypertension treated with propranolol

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We studied propranolol effect on kidney function in 10 subjects with essential hypertension and no concomitant manifest renal damage. We wanted to verify if the function was involved by the systemic hemodynamics changes induced by this drug. The patients received a diet with 100 mEq sodium per day and, after 7 days of placebo administration, were treated for 15 days with propranolol (120 mg per day). Changes in arterial pressure and systemic haemodynamics (1) and renal function (4) were followed in all of them.

A significant reduction of the arterial pressure was obtained in 6 of the studied subjects. The results obtained in "responder" are shown on the figure. From



data examination is clear that the renal function principal change is a reduced renal plasma flow (RPF). Other values are not significantly affected by propranolol. In the systemic haemodynamics it can be observed that the net arterial pressure reduction is concomitant to a 10.3% reduction in cardiac output (CO). This last result is statistically not significant and it may be secondary to the net decrease of the heart rate, that is not accompanied by evident modifications of the stroke volume (SV) and total peripheral resistences (TPR).

The slight reduced glomerular filtration rate (GFR) obtained by our group is according to that observed by Niculescu et al (8), but is in contrast with data referred by others, that did not select their studies on the base of normal renal function before treatment (5, 7, 10).

This difference may be related to the autoregulation ability of the kidney that counterbalances the flow reduction when it is still operating properly.

Reduced RPF is related with a decrease of the CO, even if this one is less important. Therefore, the reduced renal flow is probably secondary to the β -blocking agent direct action on renal system and not only related to a decreased cardiac flow. These data have been demonstrated by some authors injecting the drug directly in the renal artery (2, 3, 6).

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Malignant hypertension due to the anomaly of the left kidney, cured by nephrectomy

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Y.Y., young boy aged 13, was admitted in the clinic for his rheumatic pains. He had a traffic accident, without any serious damage.

Examination: apparently in good health.

Heart: systolic murmure in aortic area. Blood pressure: arms-260 systolic and 130 diastolic. femoral: 280-160. ECG: normal. Urine: any abnormality.

Blood: light anemia (Erythro. 3,960,000, Hgl. 70%, Leuco: 6400 serum chlor. 104 mEq. Sodium 140 in mg. Potassium: 4. I. Calcium: 5.3. Vanil mandelic acide: 19.65.

Catheterism of the kidneys: left side urine sodium 115, right side urine sodium 174 mg.

Eosin latex test: + + + +.

X ray-intravenous urography: urin excretion: right side normal, left side: diminished. Radiograms: left kidney: atrophic, right kidney: hypertrophic.

Renal arteriography: narrowing of the left renal artery with many collaterals, right side: normal. Diagnostic: malignant hypertension, cured by nephrectomy.

About the diagnosis and therapy of the so-called premature myodegeneratio cordis (on the model of dystrophia)

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Definition of the so-called "Premature myodegeneratio cordis"

First we started from the following observation. The section of the heart of 11 patients having died on 196 cases of clinically diagnosed dystrophy, in the period from July 1945, to March 1946, showed in most of the cases a brown degeneration or a fuscous atrophy, and/or periedema of heart muscle (Linzbach). Notwithstanding the fact that the average age at the time of death (56.8 years) would have led to expect a smaller frequency of pathologo-anatomical changes. Hoffmann, for instance, had found not one case of fuscous atrophy and only cases of a brown degeneration among 27 patients having died of atrial flutter or auricular fibrillation in their 5th decade. This comparison already shows that a brown degeneration or a brown atrophy of the heart muscle occurs frequently in dystrophy. We must add to this statement that in the cases first mentioned the protein level determined refractometrically was 5.85 g% (compared with an average value of 7.0 g%). For this reason, we chose the term "premature myodegeneration cordis" for the heart deformations observed in dysproteinemic hypalbuminemia. This term, however, may be applied also to the cases of dystrophy having survived, and to those where, by means of the ECG, we can find a continuing change of the curve of the heart current, even though we can no longer prove a hypalbuminosis in the chemical structure of the blood.

If we neglect the deviations of the final wave, those changes consist mainly in the decrease of the R amplitude reaching the so-called low tension. In the cases examined by us, the average value showed a maximal R amplitude of 0.75 mV in the peripheral lead II (Fig. 1). It is true, this value corresponds approximately to the average value found (according to Vaquero and Timon-Lason; Fig. 1) in cases of the same age (50 to 60 years) with a sound heart. But in the cases of dystrophy stationarily treated, we could observe a predominance of the small maximal R amplitude up to 0.6 mV.

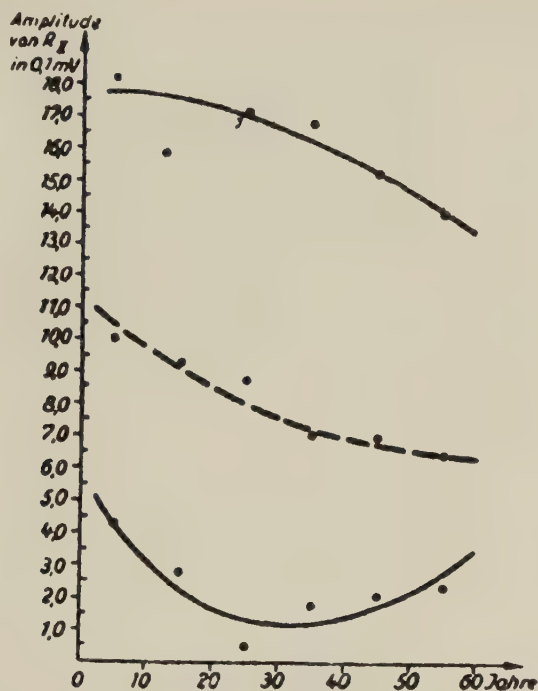


FIG. 1. Vertical: Amplitude (in 0.1 mV) of the R-wave, lead 2; Mean value — — — — — Maximum curve ————— (above); Minimum curve ————— (below). Horizontal: Age in years (health persons) (Vaquero, Limon-Lason).

This means that, provided the maximal R amplitude in lead II (Fig. 2) lies under the average curve determined by the age, we may expect a higher clinical percentage of degenerative changes in the heart muscle than usual for this age. We chose lead II in order to eliminate as completely as possible the factor of the heart position. Cases of a particular thoracal deformity, however, are to be excepted in this connection. In order to define “premature myodegeneratio cordis” in contrast to myocardosis (Wuhrmann-Wunderly) we have to state that normally a myocardosis is assumed only on condition that a dysproteinemia is found, whereas we use the term “premature myodegeneratio cordis” only when, after the disturbance of metabolism is stopped respectively the protein level of the blood is normalized again, we can prove that a damage once stated continues to exist, which we could be convinced of by comparative and check examinations on surviving cases of dystrophy.

In most of the dystrophy cases even the ECG registration became normal again. Thus it would be wrong to assume a premature myodegeneratio cordis

with these patients. It is only during the phase of acute dystrophy that a disturbance of the kation-water complex and, maybe, also of the vitamin B complex was observed which resulted in the temporary changes of the ECG. But for the smaller groups of dystrophy cases showing permanent ECG changes even after the protein level of the blood had normalized we already supposed clinically a premature myodegeneratio cordis as far, of course, as this is possible during

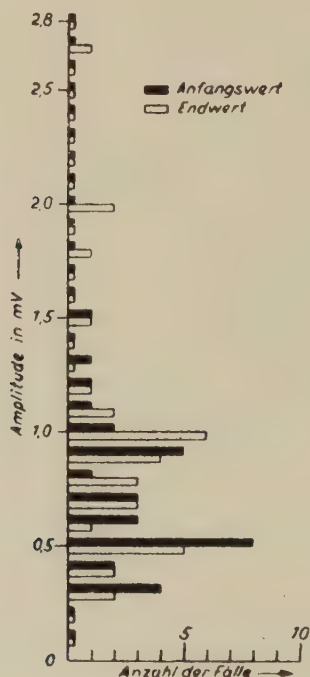


FIG. 2. Vertical: Amplitudes in mV of R II before (■) and after (□) therapy with rich protein-diet and B-complex supplies on 32 cases of patients with dystrophia (horizontal).

life. We feel compelled to make the reservation, however, that in our subject of today we only speak of the premature myodegeneratio cordis which, at the same time, we may term a partial premature degeneration including also other organic systems outside the circulation mechanism. Correlative examinations carried out on the different organic systems in order to find a premature degeneration are only of relative value. But it is necessary to get absolute values for any organic criterion objectively traceable, taking into consideration hereby the age and the variation space in a given age. If the value, in the case of a supposed premature degeneration, lies above or under the average curve, the clinical

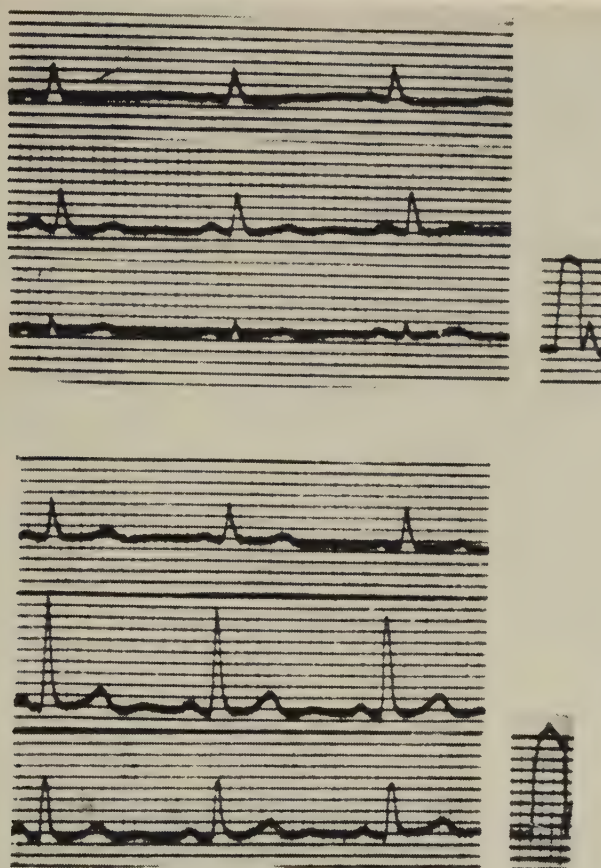


FIG. 2a. Above: ECG during Dystrophia: low voltage, of R. Below: ECG after Dystrophia: normal amplitude, of R. On the right side: calibration 1 mV = 10 mm.

assumption is justified, at least if several criteria are wanting correspondingly. The decision on the question whether the value has to lie above or under the average curve always depends upon the shape of the average curve. In case the average curve shows an ascending tendency with increasing age—as we find it, for instance, in the cholesterol level of the blood—the individual value should lie above the average curve, if a premature degeneration is assumed; whereas in the case of a descending tendency of the average curve—as we find it in the maximal R II amplitude mentioned above—the individual value should lie under the average curve.

Therapy of premature myodegeneratio cordis

The therapy of premature myodegeneratio cordis includes the therapy of latent and manifest heart failure. Normally small doses of glycoside are sufficient, as in most of the cases the reactivity on the glycosides operating on the heart seems to have increased. In contrast to the rule that digitalis or strophanthin do not act upon the normal heart, we had, by means of an electrokymographic examination of the function (pharmacological EKY test, Schennetten and Baerwald), been able to prove that—as in myodegeneratio cordis—so even in premature myodegeneratio cordis small intravenous doses of strophanthin (1/8 mg or so) had an initial effect.

The immediate effect on strophanthin which reaches its maximum 10 minutes after the injection can be traced also by means of a subtle electrocardiographical evaluation (ventricular gradient and angle delta-alpha). We reported on this already in another connection (Kanitz and Schennetten). The ECG shows also an increase of the R amplitude as a consequence of the glycoside operates pharmacodynamically (the intracardiac edema recedes, the amount of the blood decreases, the venous congestion slackens, the venous pressure sinks, and of course, the other indications of failure diminish).

It is questionable, however, whether we can, according to the definition of premature myodegeneratio cordis given at the beginning, give a reliable prognosis from the fact for a recession or an improvement of the degenerative change of the heart muscle.

For this reason we want to draw your attention to a further kind of a sole or auxiliary therapy of premature myodegeneratio cordis (any myodegeneratio cordis included). This consists in the application of heart muscle extracts containing Magnesium, Nucleoside and Adenosintriphosphate which promote circulation. These may be composed of Corhormon^R (hydrolysate extract from an embryonic calf heart), injected intramusculous or intravenous (Klingbeil).

Apart from the favourable effect on the decrease of the cholesterine level of the blood there are additionally indicated: a decompensation of heart metabolism, a promotion of the coronary circulation, and a shortening of the expulsion time.

As a criterion of the Corhormon^R effect on the ECG, we observed the tracings of the maximal R amplitude before and after a series of 10 Corhormon^R injections applied intramusculous 3 times a week in a dosis of 1 ml (Fig. 3). The cases of myodegeneratio cordis as well as premature degeneratio cordis taken at random which we gave here show in about one half of them (7 to 15) a remarkable increase of the maximal R II amplitude (up to 0.35 mV).

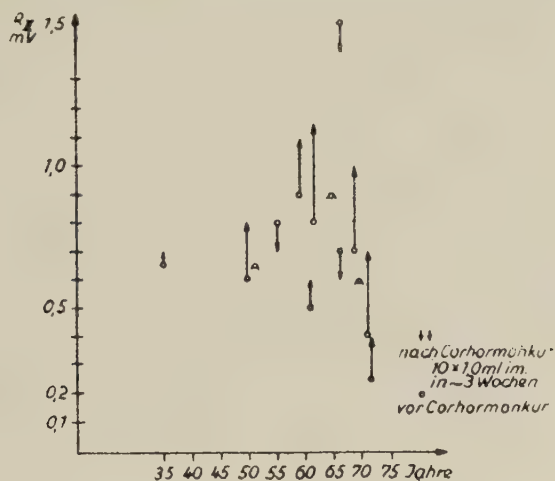


FIG. 3. Vertical: Amplitude of R II before and after therapy by Corhormone^R (10×1.0 ml during 3 weeks) on 12 patients with premature myodegeneratio cordis. Increase of the R-amplitude in 8 cases (positive effect). 3 cases without effect. 2 cases with small negative effect (decreases of R). Horizontal: Age of patients.

SUMMARY

Diagnosis: Starting from the observations of Dystrophia it accrues from the comparison of electrocardiographical with pathologic-anatomical statements that the amplitude of the R-waves (under consideration of the age-conditioned normsize of R) may be a first hint for the clinical existence of p.M.c.: the maximum amplitude of R corresponds to a peripheral absolute (less 0.6 mV) or relative low amplitude of R, i.e. the maximum amplitude is located below the age-conditioned amplitude of R. This resulted from a continuously running observation of 196 cases of Dystrophia with an average age of 52 years (aged between 25 to 77 years). At the 10 passed away patients (average age 56.8 years) the serum albumen level total was reduced down to 5.85 g%, here the absolute low amplitude of R was present. At the survivors the maximum amplitude risen from 0.7 mV up to 1.0 mV.

Pathological-anatomical finding: peri-edema of heart muscle fibres, brown degeneration, Atrophia fusca.

Therapy: protein diet and B-complex supplies. Small doses of strophantin (1/8 mg i.v.).

Moreover heart-muscle extracts (intramuscular or intravenous) power of which can be objected by rise of the maximum amplitude of R.

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Acid-base equilibrium and its relationship to natriuresis in essential hypertension

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Only a few data exist in the literature on the relationship between acid-base equilibrium and the renal metabolism of sodium in essential arterial hypertension (Arnold and Healey, 1969; Gordon *et al.*, 1970; Brautbar *et al.*, 1978). In the present study, the results obtained in unselected essential hypertensives (HT), without clinical and laboratory signs of nephropathy are reported.

MATERIALS AND METHODS

Acid-base equilibrium was determined by Astrup method in 39 HT (25 males and 14 females, aged 17-68, WHO stage I-II) and 39 normotensive controls (NT) (21 males and 18 females, aged 15-71). None of them presented hypertensive nephropathy; all treatments were stopped at least 2 weeks before the study was begun; daily dietary sodium intake was 150-250 mEq. 3 HT with metabolic acidosis (MA) of renal tubular origin (group I), 17 HT without (group II) and 8 NT underwent an urinary acidification test with NH_4Cl (0.1 gr/Kg of body weight *p.o.*).

RESULTS

A significantly ($p < 0.05$) higher incidence of mild MA was found in HT (6/39) than that observed in NT (1/39). No differences were noted in the incidences of respiratory and metabolic alkalosis in either groups. MA appear to be of renal tubular origin (normal anion gap, urinary $\text{pH} - \text{pH}_u > 5.5$ while serum bicarbonate $-\text{sHCO}_3^- < 22 \text{ mEq/l}$). In all patients, except in one of group II, the renal response to NH_4Cl was normal (minimum $\text{pH}_u < 5.4$, increase of urinary net excretion $-\text{NAE}-$ i.e. the sum of titrable acidity $-\text{T.A.}-$ plus ammonia $-\text{NH}_4^+ - \text{minus HCO}_3^-$). During the whole study in HT with MA, sodium and chloride excretion were lower than that observed in the other groups, although the difference was not significant. Urinary potassium excretion was similar in all patients. In the patient of group II, who did not show any decrease in pH_u and a blunted increase of NAE, sodium and chloride excretion were depressed during the whole period of the study. In HT with MA, NAE increased

to an extent similar to that of natriuresis (Na^+_{uV}) and it was correlated to the fraction of sodium delivered to the distal tubule, potentially exchangeable with cations ($\text{Na}^+_{\text{del}} = \text{Na}^+_{\text{uV}} + \text{K}^+_{\text{uV}} + \text{NAE}$) ($\text{NAE} = 0.444\text{Na}^+_{\text{del}} + 10.66$; $n = 10$; $r = 0.8940$; $p < 0.01$). A similar correlation was found in group II HT ($\text{NAE} = 0.173 \text{Na}^+_{\text{del}} + 48.95$; $n = 57$; $r = 0.6780$; $p < 0.01$), but it was not found in NT.

COMMENT

The existence of a significant correlation between NAE and Na^+_{del} confirms a role of sodium in the distal nephron H^+ secretion in HT, as well as in sodium depleted normal subjects (Perez *et al.*, 1977) and in cirrhotics (Better *et al.*, 1972). In fact the availability of sodium in the distal tubular fluid is a limiting factor of acid secretion (De Sousa *et al.*, 1974). A reduced availability of Na^+ in the distal nephron is suggested by the low Na^+_{uV} in HT with MA and in the patient who did not show any decrease of pH_{u} . The decrease of pH_{u} and the increase of NAE after the acid load in group II HT who had shown an alkaline urine during MA before the administration of the salt, may be linked to the more severe MA, induced by NH_4Cl administration, and to the augmented availability of Na^+ in the distal nephron, due to the natriuretic effect of the salt, since both may increase H^+ secretion.

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Pattern and incidence of ECG changes in 400 athletes - correlation with Vo_2max

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Routine medical evaluations of well trained endurance athletes frequently disclose ECG abnormalities suggesting organic heart disease (N.H. Paparo 1976). In most of the studies a small number of top athletes of the endurance fields were examined or a larger number but incompletely. In the study reported here the ECG changes in 400 amateur athletes participating in 16 diversified sport fields are evaluated and correlated to their predicted maximal oxygen uptake (Vo_2max) determined according to Astrand (1954).

The mean (\pm S.D.) age of all athletes was 21.1 ± 3.9 years (range 17-36). The mean (\pm S.D.) heart rate of all athletes was 62.1 ± 9.6 beat/min, being less than 70 in all groups examined. There were highly significant differences in Vo_2max between athletes of various sport branches ($p < 0.001$), cyclists having the highest Vo_2max ($62. \pm 9.4$ ml $\text{O}_2/\text{kgB.W.}/\text{min}$). The mean (\pm S.D.) Vo_2max in 400 athletes was 54.7 ± 10 ml $\text{O}_2/\text{kg B.W.}/\text{min}$.

$\text{SV}_1 + \text{RV}_5$ (Sokolow index) greater than 35 mm and $\text{RV}_1 + \text{SV}_5$ greater than 7 mm were found in 46.2% and 34.5% of the athletes, respectively. There was a significant correlation between these two parameters indicating a simultaneous biventricular hypertrophy. Although heart volume was claimed to be in correlation with endurance, in our material significant correlation ($p < 0.05$) was found only between $\text{SV}_1 + \text{RV}_5$ and Vo_2max , but not between $\text{RV}_1 + \text{SV}_5$ and Vo_2max . Similarly to the findings in non-athletes, a highly significant correlation ($p < 0.01$) was found between the age of the athletes and the Vo_2max .

T wave inversion, RSR in V_1 , interventricular conduction defects (IVCD), high U-wave, premature ventricular beats and terminal T negativity were recorded in 3.7, 21.2, 30.5, 90.3, 1.2 and 15.8% respectively.

Our most important finding is the high incidence of ECG changes—which were previously reported in top endurance athletes—recorded in athletes even with a relatively low Vo_2max , and in athletes of all sport branches. The significance of these changes has to be more thoroughly evaluated. Meanwhile it seems that mere occurrence of the abovementioned ECG changes in the absence

of subjective symptoms or physical finding suggestive of cardiovascular disease should be interpreted as a normal variant and not lead to restriction of physical activity. This concept is supported by the disappearance of the ECG changes following discontinuing of regular training. In each athlete a baseline ECG should be recorded as well as regular recordings to disclose any new change in pattern especially if accompanied by symptoms suggestive of cardiac disease.

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Diagonal ear lobe crease as an indicator of coronary risk factors

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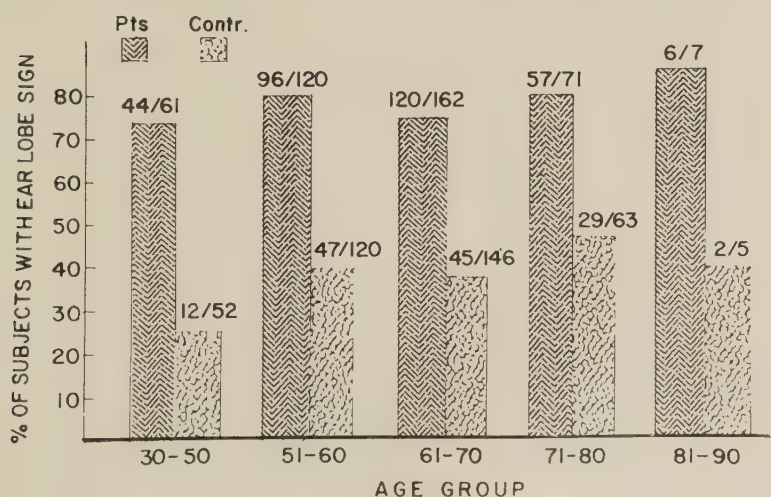
Preliminary data reported by Frank (1973) and later confirmed by others (Lichstein 1974, Doering 1977, Rhoads 1977), suggested a positive association between the diagonal ear lobe crease (ELC) and coronary heart disease (CHD).

In the present study we attempted to correlate the ELC with coronary risk factors (CRF) as well as with diabetic and hypertensive retinopathies.

421 consecutive patients with myocardial infarction (MI) and 386 control subjects without present or past evidence of CHD, diabetes or hypertension, matched for age, sex and ethnic origin were studied.

A highly significant incidence of ELC was found in patients with MI in comparison to controls (77% and 44% respectively) and in patients of Ashkenazic origin in comparison to those of non-Ashkenazic origin (80% and 64,7% respectively). Among the different CRFs, significant associations were found between ELC and hypertension ($p < 0.001$) and diabetic retinopathy ($p < 0.05$) only.

Ear lobe wedge biopsies from twelve subjects obtained after death (4 with ELC and MI, 4 with ELC but without MI, 2 without ELC but with MI and 2



without ELC or MI) revealed that significant elastic fiber tears were consistently noted in all ELC subjects and in none of the subjects without ELC. Prearteriole wall thickening was detected in the four MI patients (3 of which had ELC) and in two ELC patients without MI. It could not be detected in the two subjects with neither MI or ELC.

Our data show an increased frequency of ELC among MI patients regardless of age, among patients with diabetic retinopathy, as well as among patients with MI and hypertension. Although the number of ear lobe biopsies is small, it might be possible that a diminished blood supply to the ear lobe, a highly vascular area, may contribute to the elastic fiber tears which presents as the early creasing and folding seen by the naked eye. While the time of onset and underlying mechanism are not yet accurately determined, and its specificity has been questioned, the presence of ELC is significantly higher in patients with CHD. This easily identifiable sign, which may imply the existence of CRFs, could be reliable in screening for prospective high risk coronary patients thus motivating reduction CRFs such as smoking and hypertension, which may be instrumental in the precipitation of MI.

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Significance of arterial wall thickness in hypertension

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The commonest and most widespread arterial abnormality in hypertension is a thickening of the vessel wall, commonly called medial hypertrophy. For long this was simply regarded as a striking but unimportant result of hypertension. Recently, however, interest has been aroused by Folkow's (1) suggestion that it may be haemodynamically important, particularly in the small arteries and arterioles. Folkow conceives of the hypertrophied media as encroaching on the lumen and thereby contributing to the increased vascular resistance which is a major factor in the maintenance of a raised arterial pressure in most cases of chronic hypertension. This concept is now widely accepted (2). Recently, however, an element of doubt has crept into the concept of medial hypertrophy. Even Folkow (3) now puts the term "hypertrophy" in quotation marks and prefers to use the non-committal expression "increased wall/radius". The reason for this uncertainty lies in the fact that although the walls of the arteries appear to be thickened in relation to the size of the lumen, the presence of hypertrophy in the sense of an increase in wall mass has never been confirmed by actual measurement of the cross-sectional area of these vessels. The only studies in which such measurement has been undertaken, so far as I am aware, are those of Thomson and myself (4, 5) in man and Friedman *et al.* (6) in rats.

Thomson and I studied the arteries in the small intestine after distending them by injection into the superior mesenteric artery in eight hypertensive and twelve control subjects. We found an increased wall/lumen ratio even in arteries which were apparently fully distended, but no increase in cross-sectional area of media i.e. no hypertrophy. The increased wall/lumen ratio was due to decreased calibre. Friedman *et al.* (6) made their studies in rats made chronically hypertensive by the administration of DOCA. They measured the cross-sectional area of the media of the ventral caudal and superficial epigastric arteries. Their findings offered "no support to the view that hypertrophy of the media, often described as a feature of the hypertensive state, is either necessary consequence or an invariable concomitant of the pressure rise".

Thus, the only studies involving actual measurement of the cross-sectional area of the media in chronic hypertension have failed to confirm the existence

of hypertrophy. Furuyama (7) and his colleagues often quoted as demonstrating the existence of medial hypertrophy in hypertension, did not in fact measure the cross-sectional area of the media; they merely measured the thickness of the arterial wall when the arteries were fully distended and assumed that the increased wall/lumen ratio they found must indicate hypertrophy. This, as we have shown, is a false assumption.

CONCLUSIONS

There is no evidence that the walls of the small arteries and arterioles in hypertension are hypertrophied.

The apparent wall thickening is due to persistent contraction.

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Study of serum lipids in acute myocardial infarction with or without cardiac arrhythmias

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SUMMARY

Various serum lipids were estimated in 50 patients of acute myocardial infarction (35 with and 15 without cardiac arrhythmias), and in fifty matched controls. Mean values of serum cholesterol and triglycerides were found to be significantly raised in patients of acute myocardial infarction both with or without arrhythmias when compared to the corresponding values in the control group ($p < 0.001$). Patients with cardiac arrhythmias had significantly elevated mean serum FFA levels (Free Fatty Acids) as compared to the corresponding values either in the control group ($p < 0.05$) or in patients of infarction without cardiac arrhythmias ($p < 0.05$).

INTRODUCTION

There are conflicting reports about the level of various plasma lipid fractions in cases of Acute Myocardial Infarction (Bandopadhyaya, 1964; Dutt, 1967 and Carlson and Bottiger, 1972), and secondly there are meagre reports regarding the correlation between circulating free fatty acids and arrhythmias following myocardial infarction. Therefore, the present work was carried out to determine the levels of various serum lipids in patients of acute myocardial infarction with or without cardiac arrhythmias.

MATERIAL AND METHODS

This study consisted of 50 patients of acute myocardial infarction admitted to L.L.R. and Associated Hospitals, Kanpur and 50 cases of age and sex matched controls.

A detailed clinical history was taken and thorough physical examination was done and the cases were divided into the following groups: (1) Group A, Controls (2) Group B₁, 15 cases of myocardial infarction without arrhythmias and (3) Group B₂, 35 cases of myocardial infarction with cardiac arrhythmias.

Cases having primary or secondary hyperlipidaemias or those on antilipaeamic therapy were not included in this study. Following investigations were done in each case: (1) 12 lead electrocardiogram (2) SGOT (King, 1960) (3) Routine blood, urine and stool examination (4) Serum cholesterol (MacIntyre and Ralston, 1956) (5) Serum triglycerides (Vanhandle and Zilversmit, 1961) and (6) Serum Free Fatty Acids (Novak, 1965). Diagnosis of acute myocardial infarction was made in accordance with coronary drug project Diagnostic Criteria for Coronary Artery Disease (1973). All the patients were monitored in Coronary Care Unit for the study of arrhythmias.

OBSERVATIONS

Age and sex distribution

Out of total 50 cases in the control group there were 46 males and 4 females; they were in the age range of 31 to 76 years (mean age 53.38 ± 11.12 years). Similarly in study group there were 46 males and 4 females; they were also in the age range of 31 to 76 years (mean age 53.38 ± 11.12 years).

Site of infarction

There were 30 cases (60%) of inferior wall infarction, 19 cases (38%) of anterior wall infarction and remaining one case (2%) showed subendocardial infarction.

Lipid pattern

Mean Levels of serum cholesterol, triglycerides and free fatty acids are shown in the table.

Types of arrhythmias

Atrial arrhythmias were observed in 12 cases (34.29%), ventricular arrhythmias in 19 cases (54.28%) and A-V nodal (junctional) in 4 cases (11.43%).

DISCUSSION

Mean values of serum cholesterol in both the groups of patients of acute myocardial infarction without or with arrhythmias were found to be statistically highly significant ($p < 0.001$) as compared to those of controls. Values obtained by Dutt (1967) Patterson (1972) and Lewis (1974) are comparable with those of ours. The mean serum triglyceride levels in both the groups of myocardial infarction were found to be significantly raised in comparison to the values in the control group ($p < 0.001$). Similar rise in serum triglyceride levels have also been reported by Havel (1956) and Dutt (1967).

TABLE 1

Mean values of serum cholesterol (mg%), serum triblycerides (mg%), and serum free fatty acids (mEq/l)

	Controls (50 cases) group A	Acute myocardial infarction		Group B ₁ VS Controls	Group B ₂ VS Controls	Group B ₂ VS Group B ₁
		Without arrhythmias (15 cases) group B ₁	With arrhythmias (35 cases) group B ₂			
Serum	186.74 (±19.57)	254.8 (±45.11)	265.10 (±38.97)	P<0.001 Significant	p<0.001 Significant	
Serum	85.25 (±11.97)	120.26 (±33.43)	128.40 (±42.30)	P<0.001 Significant	p<0.001 Significant	
Triglyceride	500.82 (±111.98)	526.13 (±125.5)	671.4 (±183.03)	p>0.05 Insignificant	p<0.05 Significant	p<0.05 Significant

Mean serum free fatty acids levels in patients of acute myocardial infarction without arrhythmias were not significantly higher ($p > 0.05$) as compared to those of controls while in patients of cardiac infarction with arrhythmias mean serum FFA level was high statistically significant ($p < 0.05$) as compared to that of control group. Our findings of serum FFA level in patients of acute cardiac infarction with arrhythmias are comparable with those of Oliver *et al.* (1970) and Oliver (1972). The present study suggests that the high levels of circulating free fatty acids is related with cardiac arrhythmias following acute myocardial infarction. It is our contention that this is brought about by excessive secretion of catecholamines.

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The prognostic significance of anaemia and polycythaemia on cardiovascular mortality

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The role of haematocrit (PCV, vol. %) as a possible risk factor for cardiovascular diseases has been assessed in some studies but results have been controversial (Elwood 1974). The aims of this study were to study the association of haematocrit with established risk factors of cardiovascular disease (CVD) and with CVD mortality.

MATERIAL AND METHODS

The mobile clinic of the Social Insurance Institution carried out multiphasic screening examinations in representative population samples in various parts of Finland during 1966-1972. The present material consists of 18045 men and 16027 women in the age group 30-69, in which participation exceeded 85%. Their mortality has been followed up until the end of 1975 when the median follow-up time was 7 years. The causes of death were obtained from death certificates. When the associations of PCV with mortality was studied multiple regression and multiple logistic analyses were used to control the confounding effects of age, follow-up time, systolic blood pressure (SBP), serum cholesterol, smoking and body mass index.

RESULTS

The PCV distribution was divided into low (in men $PCV \leq 41$, in women ≤ 37), and high ranges (in men $PCV \geq 51$, in women ≥ 47). 4.4% of men and 6.0% of women had a low PCV. The prevalence of anaemia increased with age in men. In women it was highest in premenopausal age groups. 4.6% of men and 5.7% of women had a high PCV. The prevalence of high PCV increased with age.

The prevalence of high SBP, hypercholesterolemia and obesity increased with rising PCV in both sexes. The proportion of smokers was highest among men with a high PCV. In women the association of smoking and PCV was negligible.

8.7% of anaemic men, 5.1% of normal men and 10.1% of men with high PCV had treated cardiovascular diseases as judged on the basis of use of digitalis, diuretics or other antihypertensive agents. Corresponding figures for women were 6.3%, 11.7% and 24.3%. Since the associations between PCV and mortality were not materially altered when persons using these drugs were excluded, only results for all examined person are presented in the following.

Total mortality was lowest in persons with normal PCV. A *cardiovascular death* had occurred in 7.5% of anaemic men, in 4.4% of normal men and in 9.5% of men with a high PCV. Corresponding figures for women were 1.4%, 1.7% and 6.9%. The comparison of CVD risk in the high PCV group with risk in the normal group yielded statistically significant risk ratios of 1.95 for men and 2.15 for women after adjustment for the effects of age and other risk factors. 5.9% of anaemic men, 3.3% of normal men and 5.6% of men with a high PCV had died of *coronary heart disease* (CHD). The figures for women were 0.6%, 0.8% and 2.9%. The differences in men were not statistically significant. *Cerebrovascular disease* was the cause of death in 0.8% of anaemic men, in 0.7% of normal men and in 2.1% of men with high PCV. Corresponding figures for women were 0.6%, 0.6% and 2.5%. After adjustment for other factors the risk ratios were 0.74 for anaemic men and 2.74 for men with high PCV. The risk of cardiovascular death was higher in persons with high PCV than in normal persons.

DISCUSSION AND CONCLUSIONS

This study confirms the association between haematocrit level and major risk factors of CVD (McDonough *et al* 1965, Elwood 1974). Cardiovascular diseases appear to be most prevalent in persons with high PCV. Low PCV is not associated with an increased risk of CVD death but there is no conclusive evidence of a protective effect either. In men, there is no significant association between PCV and CHD death. On the other hand, there is clearly increased risk of cerebrovascular death in persons with high PCV. Elucidation of cause and effect relationships requires further study.

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The plasma kallikrein-kinin system in acute myocardial infarction

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Plasma kallikrein-kinin system is a potent vasoregulatory mechanism. Its active end-product, bradykinin, induces arterial dilatation, microcirculatory changes and pain. Because of these facts the plasma kinin system has been supposed to play a part in the different manifestations of ischemic heart disease.

We have studied the activation of this system in 65 patients with acute myocardial infarction (AMI) and 16 hospitalized control patients without infarction. The activation was investigated by measuring plasma free kallikrein, prekallikrein consumption and kallikrein inhibitory activity (Colman *et al.*, *Ann. Intern. Med.* 71, 763, 1969), and plasma kininogen consumption and kininase activity (Sipilä and Louhija, *Biochem. Pharmacol.* 25, 543, 1976) during the disease.

The activation of the plasma kinin system in patients with AMI was evident from the significant decrease of prekallikrein, with a maximum on the third and fourth day of the disease with a concomitant decrease in plasma kininogen level. The degree of the activation was correlated to the severity of the disease as judged by maximal serum total creatine kinase activity, ECG-criteria and clinical hemodynamic parameters. There was, however, no correlation with the duration of pain. No activation of the plasma kinin system was observed in the control patients.

It is concluded that the plasma kinin system is activated in the course of AMI, and that the magnitude of the changes is correlated to the extent and clinical severity—but not to the presence or duration of pain—of the infarction. Thus, the plasma kinin system may play a part in the pathophysiology of acute myocardial infarction.

The United States public health service cooperative study of the effect of treatment on morbidity and mortality in mild essential hypertension

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This study was initiated in 1966 to determine whether lowering of blood pressure by a combination therapy of chlorothiazide and whole root rauwolfia is associated with a reduction in the incidence of degenerative cardiovascular-renal complications and/or increased survival in patients with mild to moderate essential hypertension.

Patients admitted to the study were free of evidence of cardiovascular and renal complications and other known predisposing conditions. This was determined during an initial period of inpatient evaluation. All patients had an average diastolic blood pressure of 90-115 mm Hg during the sixth week of home blood pressure determinations, without antihypertensive therapy. Following three months on placebo therapy, patients were randomly assigned to the chlorothiazide (500 mg) and rauwolfia (100 mg) combination (T) or an identical-appearing placebo (P) twice daily.

Three hundred eighty-nine subjects between the ages of 21 and 55 were entered into the study. Distribution by race and sex was white males 60%, nonwhite males 20%, white females 12% and nonwhite females 8%, with average age 44 years. The average blood pressure for the group was 148.0/99.3 mm Hg. Hypertensive complications, congestive heart failure, cerebrovascular accident, left ventricular hypertrophy, cardiac enlargement, retinopathy and nephrosclerosis occurred at 18.6/100 in T compared to 44.9/100 in P. Left ventricular hypertrophy (65.3%), cardiac enlargement (22.6%), and retinopathy (7.3%) accounted for 95% of all such events. Death, myocardial infarction and other coronary heart disease occurred with equal frequency: T = 17.6/100; P = 19.4/100. Treatment failure (progressive rise in diastolic blood pressure) occurred in 20 of P and 1 of T.

Cardiovascular risk in mild uncomplicated hypertension is low. Active drug therapy does not protect against coronary heart disease or death. Systematic follow-up without drugs is a valid alternative for this large group of patients.

Cell-mediated immunity impairment in patients with bacterial endocarditis

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The immune responses were investigated in 78 patients with bacterial endocarditis. The cytologic formula of the inflammatory response induced by skin application of cantaridine, the serum fibrinolytic activity after stimulation by bismuth carbonate-pyrolidon (BCP), the induced leukocytosis and phagocytosis and the lymphocyte blastic transformation index in cultures stimulated by PHA were performed to explore the cell-mediated immunity. Serum electrophoresis and immune electrophoresis were also performed to explore the humoral immunity. Possible autoimmune phenomena were investigated Boyden passive hemagglutination test, Ouchterlony technique of double gel diffusion, Coons-Kaplan indirect immunofluorescence method for autoantibodies and Polčák phenomenon (rat mesenteric vessels constriction induced by serum autoimmune factors).

The results showed in most patients a depressed cell-mediated immunity (impaired mobilization ability of macrophages during inflammatory responses, decreased serum fibrinolytic activity before and after BCP stimulation, decreased induced leukocytosis and phagocytosis, decreased index of lymphocyte blastic transformation in culture stimulated by PHA), beside an increased humoral immune response (hypergammaglobulinemia associated with a significant increase of IgG and IgM and an increased number of bone marrow pyroninophylic plasma cells). Concomitantly, autoimmune phenomena (anti-endocardium and anti-myocardium antibodies rendered evident by Boyden, Ouchterlony and immunofluorescence test, as well as a positive Polčák phenomenon), were found.

Relationship between cardiac rate (CR) systolic arterial pressure (SAP) and watts during exercise electrocardiogram in normal subjects

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The exercise electrocardiogram was done by Elema cycloergometer. The work load increase every 3 minutes until the maximum CR (calculated by Astrand) was reached.

65 male patients were selected on following basis:

1. CR at rest was between 60 and 90/min.
2. The SAP was no greater than 160 mmHg.
3. The patients had no heart disease.

During the exercise the SAP was taken every minute during the test and afterwards until the SAP and CR returned to normal.

The following results were obtained:

1. During the exercise there was a linear relationship between the increase in CR and the increase in SAP.
2. The relationship SAP over CR variations increased with age and approached one.
3. The SAP returns to normal before the CR.
4. There is an exponential relationship between watts and $SAP \times CR$.

In conclusion the cardiovascular system adjusts itself to exercise, increasing the CR and SAP.

The SAP depends on variation of heart contractility and on peripheral resistance.

This data establishes the range of values in normal subjects.

FREE PAPERS
HEPATOLOGY

Sulfated and unsulfated serum bile acids in patients with cholestasis or liver cirrhosis

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Sulfation of bile acids (BA) has been shown to be an important metabolic pathway in liver disease (Stiehl, 1974). BA sulfates differ in metabolism, excretion and toxicity from unsulfated BA. In healthy man sulfation seems to be of great importance for the poorly water soluble and toxic lithocholic acid.

Although sulfated BA have been detected in serum, bile, urine (Makino *et al.*, 1975; Van Berge Henegouwen *et al.*, 1976) and feces of liver patients, their importance is not yet fully understood. Furthermore, because of a number of methodological difficulties encountered in their fractionation and determination, little data are presently available on the degree of sulfation and on the patterns of BA sulfates in various liver diseases.

We developed a simplified and sensitive method for the analysis of sulfated and unsulfated BA, and applied it to sera obtained from cholestatic and cirrhotic patients.

PATIENTS AND METHODS

Fasting serum BA levels and patterns were studied in 9 patients with incomplete cholestasis of various origin and in 9 patients with alcoholic cirrhosis. None of the patients studied was on antibiotic or cholestiramine therapy. None showed signs of renal disease.

Total serum BA were extracted with a batch adsorption procedure using Amberlite XAD-7; sulfated and unsulfated BA fractions were separated by column chromatography using Sephadex LH-20. BA conjugates were enzymatically hydrolyzed for 12 hours with cholyglycinehydrolase. Extraction, solvolysis and methylation was simultaneously achieved by the addition of dimethoxypropane and hydrochloric acid directly to the hydrolysis buffer. Trimethylsilyl-ether derivatives were prepared for the gaschromatographic analyses, which were performed on HIEFF-8 BP 1.0% on Chromosorb W HP DMCS.

RESULTS

Total serum BA levels were 50.7 ± 13.8 (SE) $\mu\text{g/ml}$ in patients with cholestasis, while they were 14.3 ± 2.6 $\mu\text{g/ml}$ in the cirrhosis group ($p < 0.05$). The higher levels observed in cholestasis were due mainly to the unsulfated fraction, which accounted for 38.8 ± 4.4 $\mu\text{g/ml}$ and 9.1 ± 1.4 $\mu\text{g/ml}$ respectively in the 2 groups ($p < 0.05$). The percentage of sulfation of total serum BA was $26.4 \pm 5.5\%$ in cholestasis and $31.2 \pm 6.5\%$ in cirrhosis. The degree of sulfation of the individual BA did not differ significantly between the 2 groups. Approximately 90% of cholic acid (C) was present in the unsulfated fraction, while lithocholic acid (L) was present mainly as the sulfate ester. Intermediate values were obtained for chenodeoxycholic (CDC) and deoxycholic (DC) acids.

Qualitative analysis of the serum BA patterns showed that in the unsulfated fraction C and CDC were present almost in identical proportion in the cholestasis group, while CDC was largely predominant in the cirrhosis group. DC was present in very small amounts in cirrhotic patients.

In the unsulfated fraction CDC was clearly the major BA in both conditions, while C was present in very small amounts. The relative proportion of DC and L appeared to be higher in cholestasis. In this fraction a number of unusual or unidentified BA were found, the major of which has the same relative gaschromatographic retention time as authentic 3β -hydroxy5-cholenoic acid.

CONCLUSIONS

1. The serum levels, as well as the patterns of BA in patients with cholestasis are greatly different from those in patients with cirrhosis. This difference is due almost only to the unsulfated fraction.
2. The pattern of sulfated BA is similar in the 2 conditions, the degree of sulfation increasing with the reduction of the number of hydroxyl group present in the molecule.
3. A number of unusual or unidentified compounds occur, mainly as sulfate esters, in both cholestasis and cirrhosis.

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Effect of low doses of ursodeoxycholic acid on lithogenic index of bile in gallstone patients

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It has recently been reported that Ursodeoxycholic acid (UDCA), the β epimer of Chenodeoxycholic acid, is effective in dissolving cholesterol gallstones and that low doses (150 mg/day) are at least as effective as high doses (600 mg/day) (Nakagawa *et al.*, 1977).

The aim of our study was to evaluate the modifications induced by two different low doses of UDCA on biliary lipid composition in non-obese gallstone patients.

PATIENTS AND METHODS

Twenty patients with radiolucent gallstones in "functioning" gallbladder were studied. They were randomly allotted to two treatment groups receiving in double blind 150 mg/day (11 patients) and 300 mg/day (9 patients) respectively. Dark brown concentrated bile was obtained by duodenal intubation under continuous infusion with caerulein before and after two months of treatment. Cholesterol and bile acids were simultaneously determined with a gaschromatographic method. Phospholipids were determined colorimetrically.

RESULTS

Neither diarrhoea nor hypertransaminasemia occurred during therapy. Attacks of biliary colic were less frequent in all patients.

The dose of UDCA calculated as mg/Kg/body weight was 1.9 to 3.0 (Mean 2.24 ± 0.38 SD) mg/Kg/day in the 150 mg group and 3.7 to 5.7 mg/Kg/day (Mean 4.44 ± 0.68 SD) in the 300 mg group.

Biliary bile acid composition was considerably modified during treatment in both groups. A significant correlation between the percentage of UDCA in bile and the dose expressed as mg/Kg/day was observed. Chenoid acids (UDCA

plus Chenodeoxycholic acid) showed a higher percent increase in the 300 mg group (52.5%) than in the 150 mg group (32.5%). The difference was however not statistically significant. Lithocholic acid did not increase in bile. Saturation Index (SI), calculated according to the limits of cholesterol solubility as defined by Hegardt and Dam (1971), was significantly lower after treatment in both groups. It is worthwhile pointing out that after treatment 4 out of the 11 patients in the 150 mg group already has supersaturated bile, while this occurred in only 1 out of the 9 patients in the 300 mg group.

It has recently been reported that micelles solubilize less cholesterol when the percent UDCA increases in bile. (Carey and Small, 1978). It has therefore been suggested that the SI be modified for a correction factor which would take into account the percentage of UDCA in bile. Employing this correction factor in our results, a greater number of patients (5 out of the 11 in the 150 mg group and 4 out of the 9 in the 300 mg group) still have supersaturated bile after treatment.

The percent reduction of the SI is higher in the 300 mg group than in the 150 mg group (—40.39 and —33.01% respectively). The difference is however not statistically significant. When the correction factor of Carey is employed the percent reduction of the SI is reduced in both groups.

A significant correlation between the SI and the dose expressed as mg/Kg/day has been found. When the values corresponding to the zero dose are suppressed the correlation no longer exists.

DISCUSSION

Doses lower than 5 mg/Kg/day of UDCA do not always induce desaturation of bile. If the SI is a prognostic test for the successful treatment of cholesterol gallstones (Iser *et al.* 1975), doses higher than those employed in the present study are necessary. Nevertheless 150 mg/day of UDCA induce a marked reduction of the SI of bile. 300 mg/day induce a further but not proportional reduction, thus suggesting that the correlation between the dose and the SI is better defined by a biexponential than by a linear curve. If this is the case, then it may be hypothesized that UDCA has a dual mechanism of action, for example, enhancing bile acid pool size and reducing cholesterol synthesis.

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Enzymatic system entrapped in fibres binding NH_3 in hepatic encephalopathy treatment

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The important role played by the high blood ammonium levels in the pathogenesis of acute and chronic hepatic encephalopathy had been well known for many years. Several therapeutic ways have been used to achieve a decrease of blood ammonium levels and consequently an improvement in this neuro-psychiatric syndrome: diet without proteins, some antibiotics as Neomycin or Streptomycin, surgical exclusion of the Colon, Lactulose disaccharide, Glutamic acid and Arginine, exchange blood transfusion, eterologous liver perfusion, cross circulation and, quite recently, extracorporeal circulation devices with some inert compounds, wich are able to bind blood ammonium. According to this last purpose in 12 dogs, in which high blood ammonium levels were induced by intravenous infusion of NH_4Cl (45 mg/Kg/hrs), we tested in extracorporeal circulation a by-pass (femoral artery and vein) in which were inserted polyenzimatic fibres (patented by Assoreni Biomedical Laboratories, Italy) (1, 2, 3). One gram of these fibres was inserted in the by-pass for extracorporeal circulation made of a silicone tube (height = 500 mm; internal diameter + 7 mm). The fibres were fixed at both two extremes of the tube. The silicone tube, containing

TABLE 1

NH_3 Removal rate by enzymatic system into fibres (12 dogs)

Time	0	30'	60'	90'	120'	150'	180'	210'
NH_3 removal rate: mg/hrs average)	.98	3.79	3.78	9.49	6.73	3.86	7.42	6.22
Standard deviation	.22	3.32	2.93	3.63	2.30	2.87	1.41	2.12
t								
$P < 1\% = 2.819$		2.807	0.007	4.061	2.079	2.207	2.721	1.043
$P < 5\% = 2.074$								

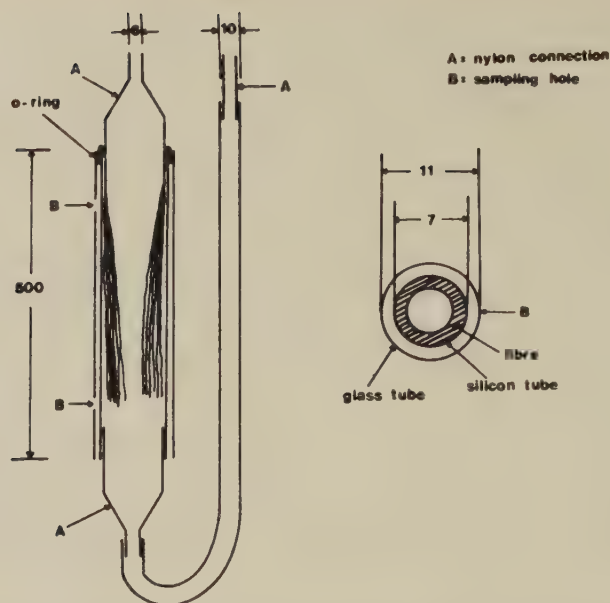


FIG. 1. Apparatus for extracorporeal circulation through reactor filled with fibre entrapped enzymes.

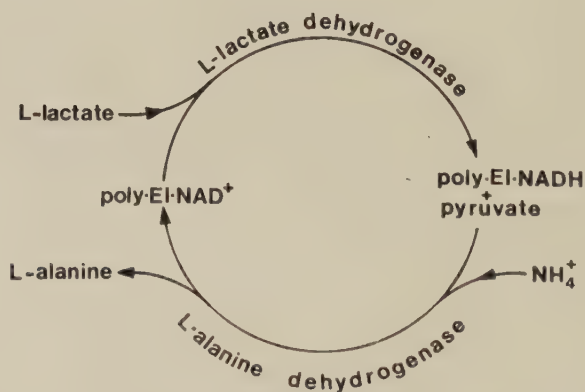


FIG. 2. L-alanine production by lactate dehydrogenase, L-alanine dehydrogenase and Poly-EI-NAD entrapped in fibres.

the fibres, was kept straight by incapsulation into a glass tube at the extremes of which two small holes allowed to take samples before and after the enzymic reaction. Connections of the silicone tube with the femoral artery and the femoral vein were made by nylon. Both the silicone and the nylon tubes were made

biocompatible by a new effective system utilizing a platelet antiaggregating agent. In fact, on the surface of the silicone and the nylon tubes there was a coating of Dithazole, which is a well known platelet aggregation inhibitor, and also fibres of cellulose triacetate were entrapping either the enzymatic system or Dithazole. No Heparin or other anticoagulant drugs were infused during all the time of experimentation. Blood flow rate was maintained constant by a peristaltic pump at about 3 ml/sec. The enzymes entrapped into fibres were alanine-dehydrogenase, lactate dehydrogenase and NAD. In the overall cycle L-alanine is produced whilst the concentration of lactic acid and ammonium are decreased.

In our experimentations the ammonium removal rate by this enzymatic system was found to be between 1 mg/hrs and 14.5 mg/hrs (average 5.52 mg/hrs \pm 3.12). The ammonium removal rate circulation was performed by the difference between the blood ammonium input and output values into the by-pass multiplied by blood flow rate/sec in the device. This removal rate was not uniform, but it continued for many hours. This finding suggests the possibility to employ this enzymatic system in the therapy of hepatic encephalopathy, but it seems to be necessary to increase its specific activity, by leading the ammonium removal rate to 20-25 mg/hrs. Finally the biocompatibility of employed materials was good; never massive thrombosis or significant decrease of blood flow rate was observed during all the time of experimentation.

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Is the adenylate cyclase/cAMP-system really involved in bile acid-induced colonic secretion in man?

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INTRODUCTION

The mechanism by which bile acids evoke fluid secretion in the large intestine has been assumed to involve the adenylate cyclase/cAMP-system (1, 2). This enzyme system is supposed to play a key role in the hormone-induced secretory process by this organ (3). However, the messenger role of this cyclic nucleotide in bile-acid induced secretion has recently been challenged by the findings of Gaginella *et al.* (4). The authors were unable to stimulate the small intestinal enzyme system with deoxycholic acid which induces a marked accumulation of water and electrolytes in this organ. The controversial results may be due to the different tissues used in these experiments. We have, therefore, studied the effects of the hormonal secretagogue vasoactive intestinal polypeptide (VIP) and the dihydroxy bile acid —deoxycholic acid—upon the adenylate cyclase in homogenates of human colonic mucosa.

METHODS

Biopsy specimens of normal colonic tissue were removed endoscopically from patients suffering from polyposis or colonic neoplasia ($n = 8$). The colon specimens were immediately placed in ice-cold Tris-HCl buffer, pH 7.4 (0.05 mol/l) and homogenized in a teflon glass homogenizer (Zell-Homogenisator, Colara-Messtechnik GmbH, Lorch, F.R.G.) after addition of 50 mmol/l tris-HCl buffer, pH 7.4, containing 3 mmol/l $MgCl_2$ and 3 mmol/l ATP. The adenylate cyclase activity was determined according the method of Salomon *et al.* (5) at 30°C. The protein content of the samples was measured according to Lowry *et al.* (6). Data are given as pmol cAMP formed per mg protein per 15 minutes. Statistical analysis was by the Wilcoxon-test for paired samples.

RESULTS AND DISCUSSIONS

Basal enzyme activity averaged 300 pmol cAMP/mg protein per 15 minutes. As described in detail elsewhere (7) human colonic mucosa contains an adenylate cyclase which can be stimulated in a dose-related manner by the hormonal secretagogue vasoactive intestinal polypeptide (VIP) (see Figure 1).

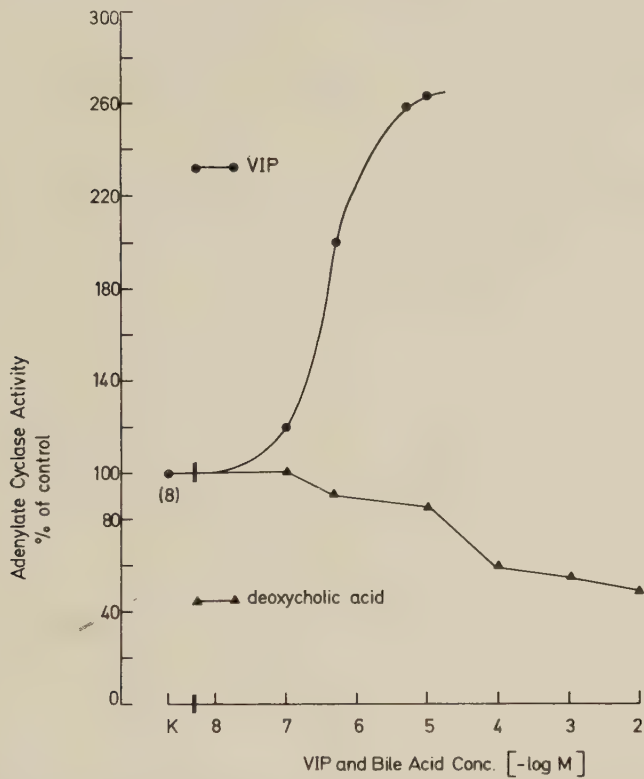


FIG. 1. Effect of ascending concentrations of deoxycholic acid and vasoactive intestinal polypeptide on human large bowel adenylate cyclase. Basal enzyme activity averaged 300 pmol cAMP/mg protein/15 minutes. Adenylate cyclase is expressed as % stimulation (and inhibition) of control (in the absence of VIP and deoxycholic acid, respectively). One representative experiment out of seven individual preparations is shown.

Stimulation by this polypeptide was detectable at 0.1 uM maximal at 5-10 uM. By contract, no dose-related activation of the large bowel cyclase was observed in the presence of deoxycholic acid (DCA) over the concentration range from 0.01 uM to 1 mM (figure 1). Concentrations of DCA above 10 uM inhibited basal cyclase. Average inhibition of about 45% occurred at a DCA concentration of 1 mM.

Our results clearly show that *human* colonic mucosa an adenylate cyclase sensitive to VIP. This neurotransmitter is known to stimulate colonic fluid and electrolyte secretion in the rat by alteration of the cyclase activity (3). Our finding support the contention that large bowel adenylate cyclase plays a physiological role in the hormone-induced secretory process in man, too. On the other side, our experiments clearly established that DCA had no activating effect on the human colonic enzyme. The data are, therefore, in contrast to the findings of Coyne *et al.* (2) for the rabbit colonic cyclase. In the latter studies, however, no clear dose-related stimulation of enzyme activity by DCA which does reflect a specific drug-receptor interaction was given. It is, therefore, tempting to speculate that activation of rabbit colonic cyclase by DCA may be regarded as a nonspecific (detergent-like) effect of this compound. The mechanism by which bile acids induce colonic secretion, and especially the role of the adenylate cyclase-cAMP system in bile-acid-induced colonic secretion in man remains, therefore, to be established.

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HB-sAg “unmasking” during immunodepressive therapy in chronic active hepatitis

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A clinical and laboratory study in 14 patients with chronic active hepatitis treated by immunodepressive drugs, is reported. They were followed up during treatment as well as 36 months after its interruption. The investigations included hepatic biopsy and serum HBsAg detection. In seven patients, the appearance of HBsAg or increase of its titer were noted and concomitant they were, for few months, favorably influenced by Therapy. The phenomenon called HBs “unmasking” is discussed from the viewpoint of its signification in the etiopathogeny of chronic hepatitis.

Predisposing factors to chronic active hepatitis

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Different factors (i.e. previous or coexisting diseases, potentially liver-toxic drugs, a genetically determined immunologic situation, etc.) can promote the evolution of an acute hepatitis to a chronic form (Gentilini *et al.*, 1971).

In order to investigate some of the predisposing factors to chronic hepatitis, we have carried out a study on the incidence of previous or coexisting diseases in 200 patients affected by chronic active liver diseases (CALD) and in their relatives, using as a control group 200 subjects without liver diseases, similar in sex and age to the ones with CALD.

HLA typing was also performed in members of 10 families in which 2 or more subjects were affected by acute or chronic hepatitis.

Our results have shown no significant differences between the group of patients with CALD and the control group, either as regards dietetic deficiencies, coffee, tobacco and alcohol consumption, or as regards the use of potentially liver-toxic drugs.

In the group of patients with CALD the association with acute hepatitis, cholelithiasis, haemochromatosis, diabetes mellitus and tuberculosis was found significant ($P < 0.01$).

Furthermore, a statistically significant higher incidence ($P < 0.01$) of cholelithiasis and chronic liver diseases was found among the relatives of the CALD patients.

On the contrary, no significant differences were found in the incidence of peptic ulcer, allergic diseases, porphyrias, malaria, brucellosis, salmonellosis, amebiasis, lues, psoriasis, gout, connective tissue diseases, chronic kidney diseases and silicosis between the CALD patients and the control group, nor in the incidence of allergic diseases, diabetes mellitus and malignant tumours between the two groups of relatives.

Our HLA typing results, restricted to a small number of families (only ten) do not allow us to draw a reliable conclusion on the prevalence of one or more specific antigens in an active chronic hepatitis.

In particular, we cannot confirm the prevalence of A1 and B8 antigens, as reported by other foreign researchers (MacKay and Morris, 1972; Lindberg *et al.*, 1975; Page *et al.*, 1975) and also of the BW35 antigens observed with great frequency in CALD patients by Italian workers (Barbanti *et al.*, 1974).

In conclusion, during chronic active hepatitis we have observed a high incidence of previous or coexisting diseases, which may either compromise the histologic structure of the liver in various ways or modify the immunological ground of the subject. The defence mechanisms are thus immunological ground of the subjects. The defence mechanisms are thus changed in such a way as to permit the establishment of other chronic diseases, especially in the liver, rich in reticuloendothelial tissue.

On the other hand, the finding of a higher incidence of cholelithiasis, and above all of chronic liver diseases, in the relatives of patients affected by CALD, suggests that genetic factors facilitate the onset of these diseases, even though our researches on the HLA system could not confirm this hypothesis.

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Disorder of the porphyrin biosynthesis during treatment with clofibrate

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A 62 years old woman presented after a protracted hypocholesterolemizing treatment with clorifibrate (2 g daily for three months) an evident disorder of the porphyrin metabolism.

Twenty-five years before the patient had been subjected to a cholecystectomy for having suffered from cholesterol cholelithiasis and during the recent years there had been observed a moderate increase in the volume and consistency of her liver.

In the course of the hypocholesterolemizing treatment she started complaining about a general malaise and after a few days her urines were of dark red colour. Later-on after exposing herself for a short while to the sun, she noticed also a redness of her face.

Clinically one observed a marked erythema on the facial skin and on the uncovered parts (neck, nape, back of the hands) and a slight succulence of the erythematous zones. The liver appeared increased in volume and consistency and painful at palpation (the lower margin exceeded the costal arcade by 6 cm, the exploratory surface being perfectly smooth). The urines were of Bordeaux red colour.

The biochemical researches permitted to observe an abundant urinary and fecal excretion of porphyrin (more than 26 mg in 24 hours), for more than two-thirds represented by uroporphyrin and for the rest mainly by coproporphyrin. One noticed a moderate urobilinuria and a moderate increase in the transaminase activities of the serum (GOT and GPT). The sero-protein pattern appeared normal; the BSF retention remained within the limits of the normal.

The clofibrate was immediately suspended (the only drug assumed in the course of the last year) and a therapy with adenosinmonophosphate (300 mg daily) prescribed with intramuscular injections, furthermore generous doses of riboflavin. At the same time the patient was advised to rest and not to expose herself to the sun.

In the course of several weeks the porphyrin excretion decreased progressively until showing normal values. The erythema disappeared rapidly. Even

after a certain time the liver remained increased in volume and consistency although it was no more painful at palpation. The serum transaminase decreased until reaching normal values.

There appears suggestive the hypothesis that clofibrate, in exerting its action on the enzymatic system of the cholesterol metabolism, might be deemed responsible for the disorder of the porphyrin metabolism observed in the patient—which besides is known to occur through the effect of several chemical substances, as it was pointed up by various AA. (1-18).

It is most probable that the pre-existing liver damage and a genetic predisposition rendered this event possible.

From the pathogenetic point of view there may be prospected that clofibrate could have interfered with the porphyrin biosynthesis, probably by exerting an inhibitory effect on the uroporphyrinogen decarboxylase or by altering the oxidoreductive hepatic systems with consequential abnormal oxidation of the uro— and copro—porphobilinogen in the respective porphyrins, in a subject with a probable genic taint and a foregoing liver damage.

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Prevalence of HBsAg, HBsAb and chronic active liver disease (CALD) in families of HBsAg or HBsAb-positive subjects

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High frequency of hepatitis B virus (HBV) infection, as indicated by positivity for HBsAg and HBsAb, has been observed in families of chronic carriers, with or without liver disease (1, 2, 3). Moreover, high incidence of HBsAg or Ab is found in CALD in countries with a large diffusion of HBV in general population (4). The purpose of this investigations is to compare the incidence of serological markers of HBV infection and of CALD in 31 families of chronic healthy carriers (CHC) and in 24 of patients with HBsAg⁺ or Ab⁺ CALD (respectively 149 and 130 members examined). Serological studies were performed by RIA (AusRIA II, AusAb). Diagnosis of CALD or CHC was confirmed by liver biopsy in all cases. Results are summarized in the tables.

TABLE 1
Overall incidence of HBsAg and HBsAb in the kindreds of CHC and of patients with CALD

	<i>Ag</i> ⁺		<i>Ab</i> ⁺	
CHC	54/149	(36.2%)	28/103	(27.1%)
CALD	50/130	(38.4%)	28/102	(27.5%)

Note. No significant differences between sexes

TABLE 2
Distribution of HBsAg and HBsAb in the relatives

	<i>Ag</i> ⁺		<i>Ab</i> ⁺	
Husbands and wives	3/42	(7.1%)	19/29	(65.5%)
Siblings	13/75	(17.2%)	11/49	(22.4%)
Brothers	24/56	(42.8%)	9/39	(23.1%)
Parents	6/26	(23.1%)	9/17	(52.9%)
Others	7/25	(28.0%)	4/16	(25.0%)

Note. No differences between males and females, and families of CHC and CALD

TABLE 3

Overall incidence of CALD in the kindreds of CHC and of patients with CALD

	CHC		CALD	
HBsAg ⁺	2		36	
HBsAb ⁺	2		5	
Negatives	3		1	
TOTAL	7/149	(4.7%)	42/130	(32.3%)
Males	3/68	(4.4%)	24/64	(37.5%)
Females	4/81	(4.9%)	18/66	(27.3%)
Additional cases of CALD not tested for Ag/Ab	2		4	

TABLE 4

Probability of CALD in the relatives of CHC and CALD

CHC	7/118	(5.9%)
CALD	18/106	(16.9%)

CONCLUSIONS

1. Diffusion of HBsAg/Ab is identical in the families of CHC and of patients with Ag/Ab-positive CALD, showing a similar endemic-epidemic situation. HBsAg is more frequently found in brothers of the probandi, whereas HBsAb is remarkably more frequent in husbands and wives. 2. General incidence of CALD is significantly higher in the kindreds of patients with CALD (particularly in brothers and parents) than in those of CHC. 3. These results suggest that, beside HBV infection, other factors (genetic, immunologic etc.) co-operate to the pathogenesis of CALD, also in population groups with high diffusion of HBV.

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Blood volume as reference parameter in diuretic therapy of cirrhosis

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One of the most interesting features of hepatic cirrhosis is the establishment of the hypertension in the splanchnic district with no variation of systemic pressure. This could be due to the decrease of the flow and to the slow-down of flux within the district with rise of intravascular pressure and displacement of liquid (blood) toward this district, until the rise of pressure is balanced by a deformation of the involved vascular system. Portal hypertension and hypoproteinemia are the main factors which cause ascites formation, together with others like district capillary, hormonal and renal abnormalities. Therefore, the presence or formation of ascites is closely related to the ratio between colloid-osmotic and intravascular pressures of the portal system with reference to intrasplenic pressure.

The reabsorption of ascites and decrease of intrasplenic pressure can be achieved referring to possible haemodynamic conditions through: *a*) decrease of venous pressure; *b*) reducing blood pressure and the gradient of systemic pressure; *c*) variation of hydrostatic pressure with changes in body position (in this case no reabsorption could be obtained). The first condition can be obtained clinically by decreasing the blood volume and particularly the venous blood volume through a diuretic therapy. The decrease of the venous blood volume reduces the venous pressure and can influence the blood pressure and the gradient of systemic pressure. However there are disadvantages to this therapy, such as the decrease of portal (splanchnic) circulation and of effective renal flow, which could produce an irreversible hepatic-renal syndrome.

Blood volume is the determining parameter to point out periferic circulatory failures able to influence systemic circulation. We refer to blood volume in relation to metabolic hepatic flow and glomerular filtration rate to define the limits of interruption or reduction of the diuretic treatment. Also the choice of diuretic drugs, which usually are used in association, should be oriented to those diuretics that prevalently cause a water diuresis and refer to free water clearance. In our experiments we have examined patients with hepatic cirrhosis with ascites. All the patients have submitted to the following clinical determinations: I) Clearance of B.S.P. by determination of hypovolemia; II) Clearance of creatinine for glomerular filtration rate; III) Clearance of blood urea and

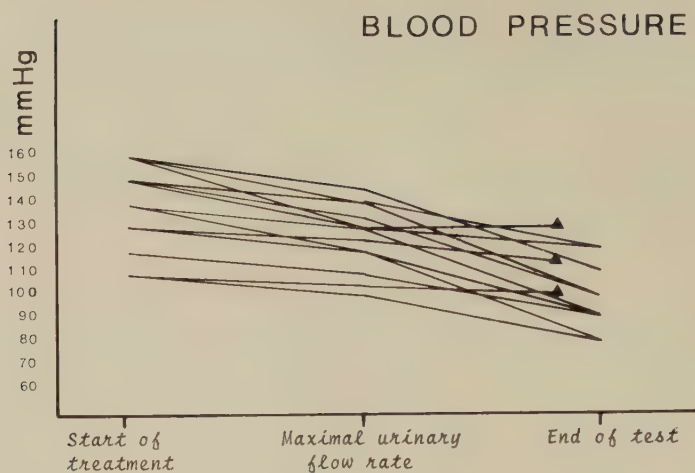


FIG. 1

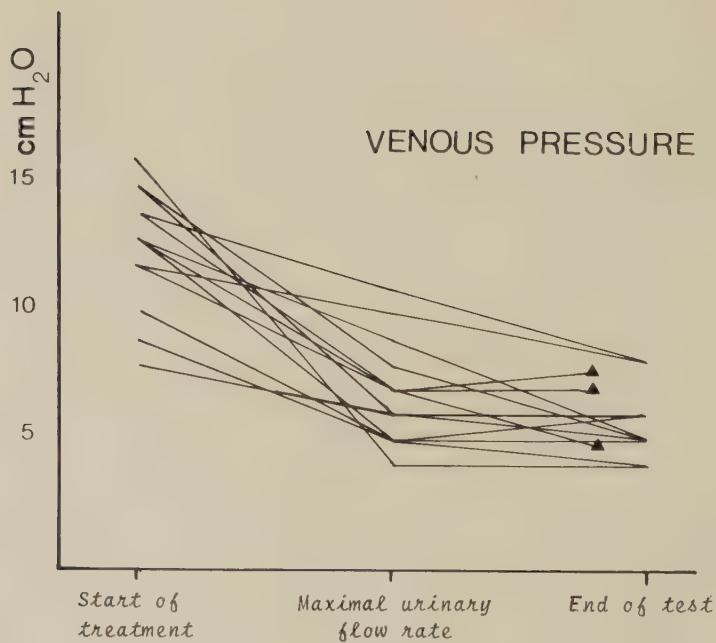


FIG. 2

uric acid for evaluation of renal function; IV) Venous pressure as index of volemic changes; V) Blood pressure: as parameter of a change of the gradient of systemic pressure; VI) Clearance of natremia and kalemia for the evaluation of free water clearance; VII) Clearance of colloidal gold to determine the metabolic hepatic flow. After this determination the patients were administered associated diuretic drugs. In the course of diuretic treatment the patients were submitted every day to determination of blood pressure and venous pressure. The decrease of venous pressure, but mainly a decrease of blood pressure, were assumed as significant parameters showing a periferic circulatory failure. Fig. 1-2 report the diagrams of venous and blood pressure versus time. The determinations of blood volume, glomerular filtration rate and metabolic hepatic flow were effected: I) at the beginning of treatment or in course of treatment; II) after three days by the induction of a maximal urinary flow; III) every five days till the first evidence of impairment of filtration glomerular rate or of metabolic hepatic flow or both. In Fig. 3-4-5 the behaviour of the above mentioned pa-

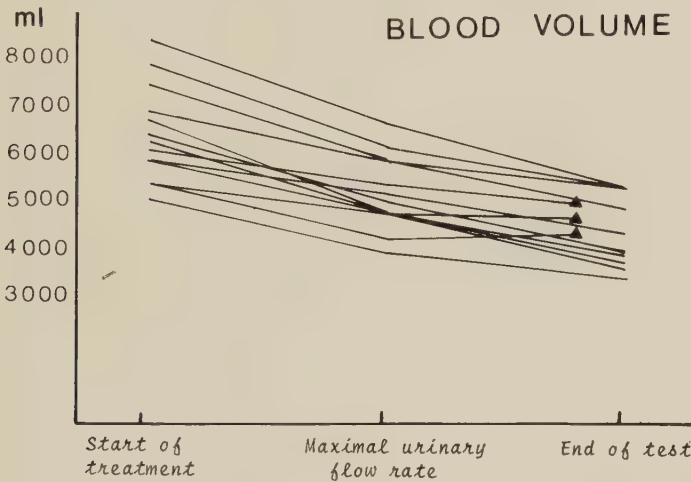


FIG. 3

rameters is shown before the treatment, after three days by increase of diuresis and when the treatment was ceased. In Fig. 6 the behaviour of clearance of free water is illustrated. The patients who had a total reabsorption of ascitic liquid were submitted to the same determinations. In fig. 7 the blood volumes of patients who still had ascitic liquid at the end of treatment and patients with hepatic cirrhosis with total reabsorption are compared. By the analysis of the results we can point out some significant observation. The blood volume seems

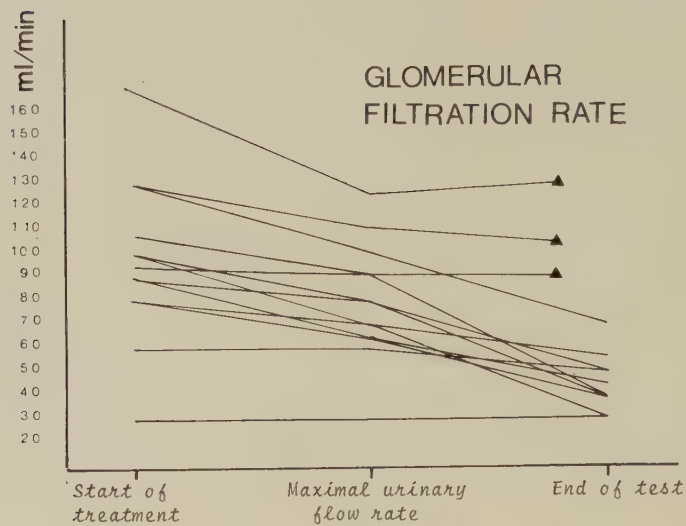


FIG. 4

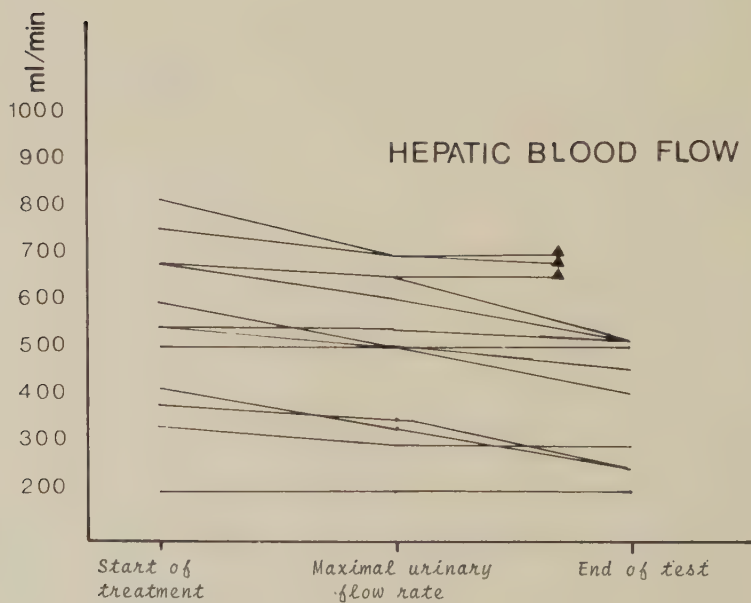


FIG. 5

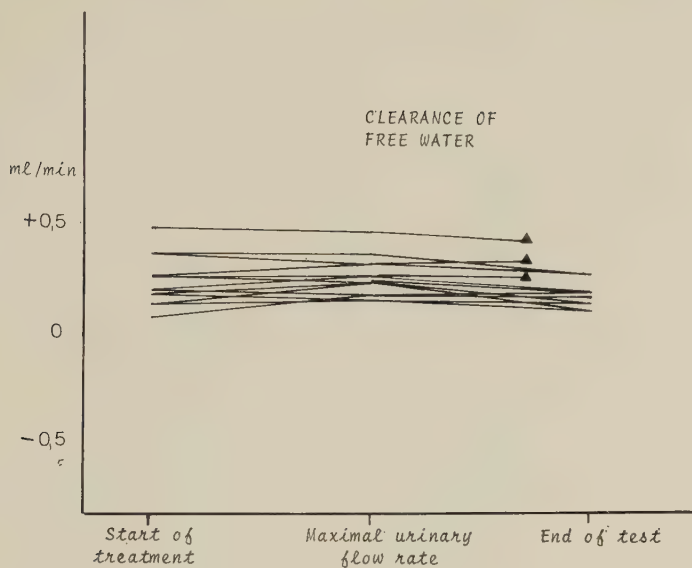


FIG. 6

to be the most important parameter to control the diuretic therapy since it decreases proportionally to the increase of diuresis. Also the venous pressure has similar behaviour, although different from that of blood volume, since it tends to lower steeply even before blood volume starts to reduce. It should be kept in mind that venous pressure is a function of another variable: the vascular tone. Moreover the blood volume, even if it decreases progressively with the continuous diuretic treatment, does not alter the metabolic hepatic flow and the glomerular filtration rate. Before this condition occurs, there is a therapeutic margin corresponding to the reduction of 15/20% of the initial blood volume. At this point a progressive decrease of blood pressure instaurates. Obviously changes in blood pressure, with no corresponding changes in blood volume, are not a conclusive evidence to reduce or interrupt the diuretic treatment, since the blood pressure depends on several different parameters, such as the after load and the myocardic tone. In patients who had a total reabsorption of the ascitic liquid the volemia was decreased even if moderately. The initial blood volume of these patients was inferior to the blood volume of the patients with ascitic liquid that resulted unmanageable. This proves a correlation between hypervolemia and intravascular splanchnic pressure as a resultant of only element: the district vascular resistance. It is difficult to state a correlation between splanchnic hypertension and the beginning of an unmanageable ascite since it would be convenient to determine first for every patient the connection between splenic

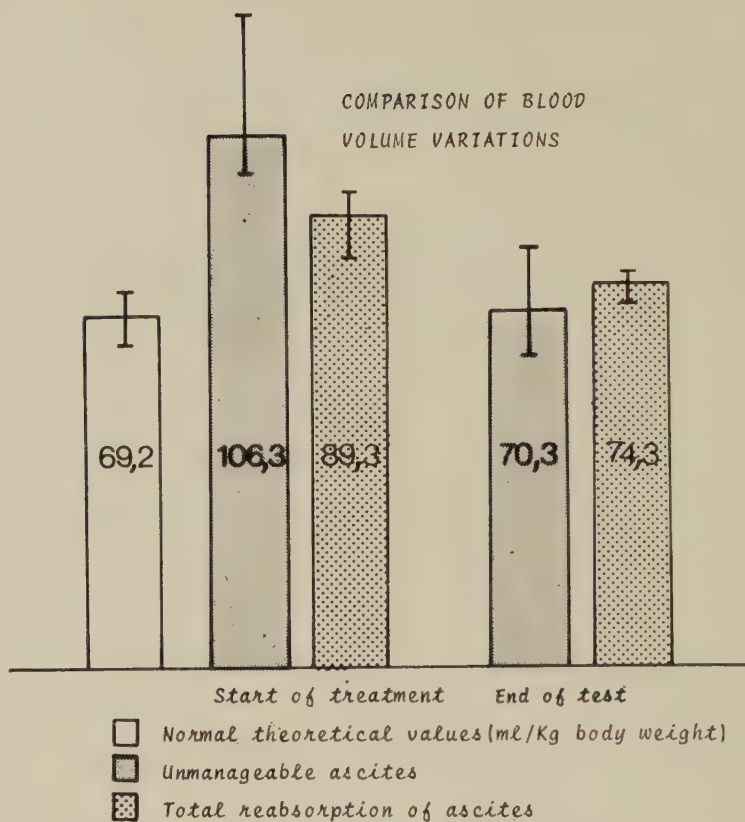


FIG. 7

pressure and oncotic pressure. Moreover other district conditions are the alteration of capillary vascular system and consequent oncotic district changes seem to be of relevant importance. In a patient, who had hemorrhagic complications it was observed that the reduction of the corpuscular mass was rapidly balanced by the increase of the plasmatic blood volume. There is good evidence that ascite is unmanageable when the reserve of interstitial fluids lowers thus producing a drop of the blood volume.

Non-specific reactive chronic hepatitis and opportunity of turning the same through immunoaggression into a form of chronic aggressive hepatitis

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There have been carried out studies, clinically, biologically, immunologically, and morphologically (liver biopsy puncture, hepatosplenic scintigram) in a number of 68 cases with hepatic involvement of the type nonspecific reactive chronic hepatitis.

The form of nonspecific reactive chronic hepatitis, we isolated morphologically from other forms, affects especially groups of ages between 31-60 years, being more frequently found with males, 65%.

Nonspecific reactive chronic hepatitis includes histologic modifications, generally slight, of nonspecific aspect and predominant inflammatory reaction of the liver, more frequently when the same is involved during chronic digestive affections (those of the biliary tract, duodenal ulcer, resected stomach) and following pleuropulmonary tuberculosis.

This form of chronic hepatitis manifests on clinical and biological area, through signs, symptoms and biochemical modifications, suggesting a lowgrade hepatic involvement.

Recognition of nonspecific reactive chronic hepatitis is a complex process, needing correlation of data from anamnesis, clinical and laboratory-biochemistry, immunology-including liver biopsy puncture. To individualize this form of chronic hepatitis means a reality.

Presence, relatively frequent, of histologic lesions, of hepatocellular focal necrosis brings into discussion a possibility of turning this form of chronic hepatitis (subsequent to appearance and evidence of immuno-aggression factors) into an active form, of aggressive chronic hepatitis.

Consequently, a periodic, medical, complex control is imperious in order to detect this eventuality and, in that case, to fix corticosteroid and immuno-suppressive therapy.

Serum T agglutinin in subjects affected by hepatic cirrhosis or other liver disease

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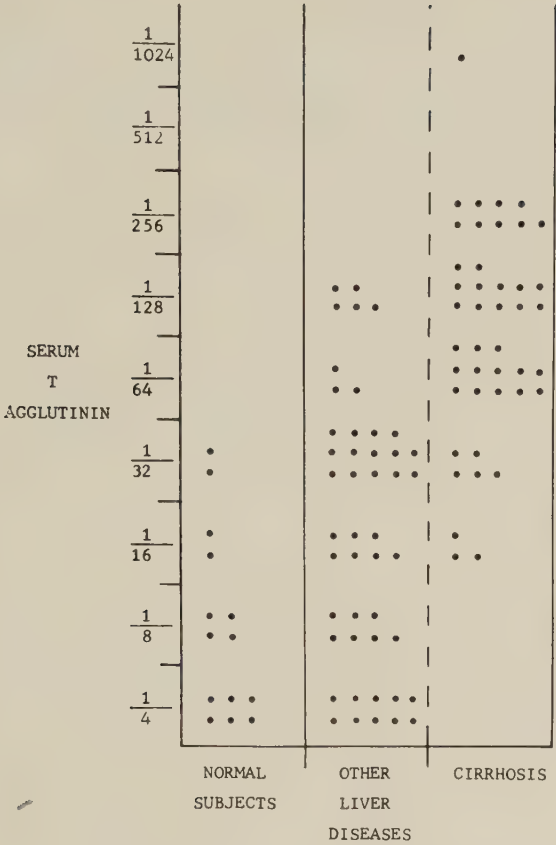
From the research undergone in recent years, it has been found in various diseases of the liver, the existence of a complex immunitary movement, whose function in the pathogenesis of the liver diseases in which it has been observed, is not yet fully appraisable today. Notwithstanding these reserves, there is no doubt that the positivity of some immunological phenomena can contribute to the nosographic identification and to the differential diagnosis of these affections.

Following these studies, after having considered the behaviour of the anti-gamma globulin factors, the antinuclear factors, of the auto and hetero antierythrocyte antibodies and of the immunoconglutinin (1), our attention has been drawn by the observation that an increased tendency is present in the synthesis of hemoagglutinins, in relation to an isoantigen stimulation, in the hepatic cirrhosis (2). In the sphere of hemoagglutinins we have taken into consideration the anti-T agglutinin by which occurs the agglutination of erythrocytes pretreated with neuroaminidase which activates the cryptantigen T. In fact this anti-T agglutinin, which according to a unique and partial signalling of the Italian literature (3) can be increased in the hepatic cirrhosis, does not result as electively studied in the liver diseases.

METHODS

The method of determining the anti-T agglutinin consists of the study of the agglutination of a standardised suspension of a pool of erythrocytes group ORh D +, incubated before with neuroaminidase, by the serum of a patient in the examined group, confronted with normal serum (3).

As a parallel, the study of the T antigen and of its "strength" is based on the agglutination of the erythrocytes activated with neuroaminidase by a lectin anti-T extracted from *A. Hypogaea* (4).



RESULTS

The results reveal that there is a significant statistical increase in the anti—T agglutinin in 43 patients with hepatic cirrhosis, in phase of ascites, while in 19 with acute hepatitis in icteric phase HBsAg + or—, 12 with cronic hepatitis, 6 with first stages of Banti’s Sindrome, 3 with colostatic icterus and 6 with hepato-colangithis, there is no significant deviation from the norm. As a parallel to these variations of the anti T agglutinin, no modifications of the “strength” of the T antigen have been observed in 18 subjects with cirrhosis.

CONCLUSIONS

From these observations it is clear that the increase of the anti—T agglutinin seems to be a characteristic of the cirrhosis and not of other hepatic affections.

The mechanism of the increase of the anti—T agglutinin is not yet clear. If one wishes to admit that the titre of the anti—T agglutinin is maintained, in the same way for anti A and anti B, from a subliminal stimulation by the T antigen, the activity of other mechanisms (major revelation of the cryptoantigen?) must be expected, given that the “strength” of the T antigen in cirrhosis subjects results normally.

The hypothesis that the increase of the anti—T agglutinin is provoked by the circulation in cirrhosis subjects, by the system of anastomosis portacava, of antigenic substances of microbic origin having determining points in common with the T antigen is worthwhile studying.

It can be expected finally, that the increase of anti—T agglutinin should be inserted in the complex of the immunological hyperreactivity postulated in cirrhosis subjects, in the sphere of the increased tendency of the formation of isoemoagglutinins observed in these subjects, the mechanism and significance of which are however obscure.

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Acid-base balance and urinary hydrogen ion excretion in patients with liver cirrhosis on the diet with "adequate" protein content

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Protein content in the diet of patients with liver cirrhosis is evaluated as "adequate" when assures slightly positive nitrogen balance. In the most of cirrhotic patients 1.0- 1.5 g of protein/kg body weight/24 hrs were recommended.

The aim of this study was the evaluation of the effect of such a diet on acid-base balance and urinary hydrogen ion excretion in cirrhotic patients. Eight cases of liver cirrhosis were studied: 4 compensated and 4 decompensated. The investigations were carried out during determination of nitrogen balance. All patients received "adequate" protein diet for at least 6 days. Blood pH, $p\text{CO}_2$, bicarbonate as well as pH, titrated acidity, ammonia and bicarbonate excretion in 24 hrs urine collections were measured daily by commonly used methods. In patients with compensated liver cirrhosis transient rise of respiratory alkalosis was observed. Urinary excretion of hydrogen ions rose evidently, mainly in the form of ammonium ions. The features of renal compensation of respiratory alkalosis were absent. In patients with decompensated cirrhosis an increase of protein intake caused persistent, more pronounced respiratory alkalosis with a fall of serum bicarbonate level which was not caused by urinary bicarbonate wasting. Urinary hydrogen ion excretion moderately rose. In 2 cases from this group a considerable fall in creatinine clearance with a fall of urinary hydrogen ion excretion occurred and the features of metabolic acidosis developed.

It may be concluded that "adequate" protein diet in liver cirrhosis causes distinct disturbances of blood acid-base balance. Renal compensation of developing respiratory alkalosis was not observed.

Partial hereditary hepatic insufficiency (P.H.H.I.) Symptoms - Treatment

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For many years, I have verified that only certain persons suffering from a P.H.H.I. which can be known or unknown by themselves, may present during their life time one or several of the following symptoms, which often manifest themselves for the first time after an emotional shock: epigastric pain located at the coeliac plexus, asthma, spasmodic rhinitis and tracheitis, hay fever, psoriasis, vitiligo, itchings, haemorrhoid crisis, excessive anxiety, uncontrollable hiccups sobs or sneezing, dental arthritis, muscular pains in arms or legs, cramps, thoracic pains, bouts of fever without any evident organic cause, hepatic and nephritic colics, hepatic diabetes, allergies provoked by pollens, feathers, animal hair, odours, plants, dusts, medicines, allergies to certain kinds of food or drink (more and more frequent since chemical products have been used in agriculture and livestock), aphthas, occupational allergies (they appear only in a very small percentage of manipulators of these products under suspicion, they are not therefore occupational illnesses). The same allopathic therapy is successful with all allergies while useless and sometime dangerous tests followed by specific desensitization rarely cure them and should be abandoned.

The treatments of all those illnesses before referred and which are in reality only P.H.H.I. symptoms consist in daily intravenous injections for a period of twenty days or so of original medical preparations composed of several well known salts used for a long time in pharmacy and well defined chemically. These salts acquire new therapeutic powers because of their association through a synergic mechanism. The innocuity and efficacy of these preparations has been checked in a Paris hospital during eighteen months, except the one of psoriasis and vitiligo. These treatments do not require absence from work except for very grave conditions and tolerance is never observed. They do not need the use of steroids nor any of their derivatives. Three years after disappearance of P.H.H.I. symptoms treated with these preparations, I have counted about thirty per cent of recurrences which disappear at once with renewed treatment.

I have noticed for ten years that 319 patients treated for cancer by oncologists had suffered before from P.H.H.I. symptoms either treated with no success or not treated. I have observed on the other hand, in 12 cancer patients whose P.H.H.I. symptoms had been treated with success, with my original preparations an unusual stabilisation of their cancer, a quite good general state of health, rare occurrence of metastases an unusual prolongation of life.

Enzymatic diagnosis of hepatopathies

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The liver, because of its morphological and physiological characteristics, is an organ rich in enzymes. Studies of homogenized liver preparations have made it possible to learn that the mitochondrias of the hepatocyte, more abundant on the periphery than in the centre, contain four enzymatic systems capable of causing, under certain conditions, hydrolysis of the ATP at different pH values: 9.4; 8.5; 7.4; and 6.3.

In the mitochondrial matrix are found the enzymes of oxidation of the long chains of fatty acids, with intermediate formation of a coenzyme, fatty acid, and acetyl coenzyme A.

Also mitochondrial are the following: glutamate-dehydrogenase (GLDH) to the extent of 100%; the alkaline phosphate (PHK) 50%; the aspartate-amino-transferase (AST formerly GOTO 40%; and alanine-amino-transferase (ALT formerly GPT), α -hydroxy-butyrate-dehydrogenase (α -HBDH) and pseudocholinesterase (CHT) 10%.

In the lysosomes we found catalase, esterase, and xanthinoxidase. In the smooth reticulum opposite the membrane γ -glutamyl-transpeptidase (γ -GT), and in the rugous reticulum pseudocholin-esterase (CHT). In the cellular membrane alkaline phosphatase (PHK) and the alanine and aspartase amino-transferases, sorbite-dehydrogenase (ICDH) and 5. ND.

When hepatic activity increases the serum levels is raised because of reduction of synthesis capacity of the cell and greater impermeability of the membrane. All these enzymes have been studied in different hepatic processes.

MATERIAL AND METHOD

Patients from the oncology, digestive, and internal medicine polyclinics. Determinations were made before treatment, blood being taken from the basilic vein without compressor, the patients not having taken food for 12 hours.

The enzymatic determinations were made using the Optimized Methods of the firm Boehringer-Manheim de Espana, and 5. ND using the Kit obtained from the Sigma Chemical Company of St. Louis, U.S.A.

Readings were taken on Unicam-SP-850 with bath thermostatically controlled at 25° C.

TABLE 1
Serum enzymes

	High	Moderately high	Small high or normal
Alcoholic hepatitis (22)	γ -GT (800) ICDH \ddagger (19) MDH (158) ALT (48)	α -HBDH (197) AST (30) CHE (2100)	LAP (18) SDH (0.5) GLDH (3.5) S'ND (0.8) ALK-Ph (198)
Viral hepatitis (35)	γ -GT (500) ALT (52) CHE (300) AST (48) GLDH (18) ALK-Ph (282)	SDH (1) MDH (118) α -HBDH (224) LAP (68) ICDH (11)	5'ND (1.2)
Chronic hepatitis persistent (36)	ALT (92) AST (56) SDH (2)	γ -GT (36) α -HBDH (188) ICDH (11) MDH (118) CHE (1,700) ALK-Ph (220)	GLDH (4.5) 5'ND (0.9) LAP (42)
Chronic hepatitis aggressive (23)	γ -GT (140) AST (164) ALT (178)	GLDH (9.5) CHE (2, 100) LAP (72) ICDH (13) SDH (1) α -HBDH (207) ALK-Ph (224)	MDH (98) 5'ND (1.41)
Cirrhosis liver (21)	AST (71) SDH (2) ALT (55) LAP (98)	γ -GT (52) GLDH (7.5) α -HBDH (214) ALK-Ph (234)	CHE (3, 100) MDH (98) 5'ND (0.9) ICDH (9)
Primary carcinoma liver (29)	LAP (120) GLDH (22) SDH (10) AST (73) ALT (59) ICDH (27) 5'ND (6.8)	γ -GT (80) CHE (1, 100) MDH (128) ALK-Ph (260) α -HBDH (274)	
Metastatic liver (29)	LAP (108) AST (69) ALT (51) SDH (3) 5'ND (5.4)	GLDH (12) CHE (1, 100) MDH (118) γ -GT (60) ICDH (13) ALK-Ph (220) α -HBDH (264)	

TABLE 2

	AST/APT	$\frac{AST+APT}{GLDH}$	$\gamma\text{-GT}/AST$
Alcoholic Hepatitis	0.9-4.0	< 50	< 2
Viral Hepatitis	0.1-1.0	< 50	> 2
Chronic Hepatitis	According to course	20-50	According to etiology
Obstructive Icterus	0.2-2.0	> 20	< 2
Biliary Cirrhosis	0.6-1.8	> 20	< 5
Metastatic Liver	0.9-5.0	> 15	< 20

RESULTS AND DISCUSSION

Table 1 shows the different hepatic processes, the numbers below indicate the patients studied. Beside each enzyme we find the average values. We can distinguish the difference of the average of each enzyme in each hepatic process, and we have been able to establish a table of values that aids diagnosis. In Table II we explain the values that use of the quotients gives us. These are very useful for differential diagnosis of icteric hepatopathies. In the case of the majority of these patients a pathologico-anatomic study was made, confirming the enzymatic diagnosis, which made us reach the conclusion that enzymatic diagnosis is of value, avoiding in many cases the use of surgical methods.

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Study of the alternative pathway of the complement in chronic liver disease

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In previous papers we have studied years ago the behaviour of the third and fourth fractions of the serum complement in different liver diseases, coming to the conclusion that the drop in the C'3 and C'4 was linked with the functional state of the liver and that, therefore, the hypocomplementemia seemed, in principle, to be due to a synthesis defect in both fractions of the complement, rather than to its consumption.

However, in a certain percentage of the cases studied, we observed that while the drop in the C'3 continued, the C'4 was absolutely normal, in the same way as other hepatic synthesis proteins (albumin, prothrombin, fibrinogen). In view of this, we have studied the alternative pathway of the complement, to be specific the C'3 proactivator and B factor of the properdin (C'3 PA).

MATERIAL AND MÉTHOD

We have studied 29 cases of chronic liver disease (5 chronic active hepatitis, 12 alcoholic cirrhosis and 12 postnecrotic cirrhosis). The clinical history, biochemical and endoscopic data and liver biopsy were taken into consideration for the diagnosis. The different fractions of the complement (C'3, C'4 and C'3 PA), were measured by radial immunodiffusion in agar, using Behringwerke immunoplates.

RESULTS

From the results obtained, we have separated those cases which presented low seric levels of C'3 PA, with the result that they were all accompanied by very low seric levels of C'3, with normal C'4 readings. We call this Group I. Group II consisted of patients with normal C'4 and C'3 PA and only a slight drop in C'3. The following chart shows the results obtained in both groups and the control.

<i>No. Patients</i>	<i>C'3PA</i>	<i>C'4</i>	<i>C'3 (mg%)</i>
Group I 14	8.64 \pm 3.95	29.57 \pm 11.21	57.50 \pm 16.64
Group II 15	17.60 \pm 5.40	39.41 \pm 19.02	83.73 \pm 23.32
Control	16.40 \pm 2.94	25.00 \pm 5.00	145.00 \pm 35.00

DISCUSSION

The results which we have obtained do not enable us to make decisive conclusions. It is perfectly clear, as we have suggested in previous papers, that there are two, well defined groups of patients: one which presents low C'3 and C'4 which would suggest either an activation of the classical pathway of the complement, or more probably, as we have stressed, a synthesis defect in the liver due to the deteriorated, functional state of the liver. On the other hand, there is another group of patients, clearly distinguished from the previous one, by virtue of the existence of a normal C'4 with a noticeable drop in the C'3 and C'3 PA, which rules out the existence of a participation of the classical pathway of the complement and which cast doubt, on the other hand, on the synthesis defect, as this would take in the three proteins studied and not just the C'4.

This calls into discussion if, as happens in other illnesses (membrano-proliferative glomerulonephritis, for example), there is an intervention of the alternative pathway of the complement in such cases of hepatopathy or whether, on the contrary, this is a detail of dubious value and difficult interpretation. It is clear that the results obtained lead us to assess the possibility of the existence of an activation of the alternative pathway of the complement, with C'3 PA consumption, rupture of the C'3 molecule with passing to C'3b and thus continuing the flood of C'5, C'6, C'7 reactions, etc. with the resulting drop in the C'3 reading in the serum and normal ones for C'4 which is not involved in the reaction.

However, in order to be able to confirm all this, it would be necessary to show in the serum of these patients the activated C'3 and C'3 PA fractions, this being the aim of our current research.

Molecular pathology of hepatic porphyrias

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In porphyrias including lead intoxication there are relative enzyme defects in the porphyrinogen and heme synthesis pathway (1). Each hepatic or erythropoietic porphyria is characterized by a primary partial enzymatic defect, which produces also compensatory mechanisms over regulating processes in the heme synthesis chain. The acute hepatic porphyrias are essentially regulatory disorders at the molecular level and have several features in common: drug idiosyncrasy, premenstrual manifestation, clinical syndrome with abdominal, cardiovascular, neurologic and psychiatric symptoms, enhanced excretion of porphyrins and their precursor (δ -aminolevulinic acid and porphobilinogen) in typical constellations in the urine, elevated porphyrin excretion in the stools (in variegate porphyria and hereditary coproporphyria and occasionally in acute intermittent porphyria), enhanced inducibility of hepatic δ -aminolevulinic acid synthase due to partial enzyme defects in the biosynthetic sequence (uroporphyrinogen synthase, coproporphyrinogen oxidase and ferrochelatase, favorable response to treatment with glucose, propranolol, and hematin, and development in a four-stage progression: phase of the genetic defect \Rightarrow latency phase (compensated \Rightarrow decompensated) \Rightarrow clinical manifestation).

The control of porphyrin synthesis through heme on δ -aminolevulinic acid synthase seems only of relevance in the liver. Neuropsychiatric symptoms are associated with the extreme elevation only of the porphyrin precursors but not of that of porphyrins. Porphobilinogen is not transformed equimolarly to uroporphyrinogen. Thus, uroporphyrinogen synthase is an important limiting and controlling factor in porphyrinogen synthesis. Increased porphyrin synthesis in acute intermittent porphyria is a sign of compensation of the diminished uroporphyrinogen synthase activity. In clinical enzymology the two cytosolic enzymes uroporphyrinogen synthase in acute intermittent porphyria and uroporphyrinogen decarboxylase in chronic hepatic porphyria have reached diagnostic importance: they both can be determined in liver and red cells.

Not only hereditary hepatic porphyrias of the acute forms, but also chronic hepatic porphyrias which are always accompanied by chronic liver damage, exist in clinically latent and manifest stages. Chronic hepatic porphyrias can be differentiated on the base of the characteristic urinary porphyrin excretion and

the accumulation of uro- and heptacarboxyporphyrin in the liver. A diminished activity in hepatic uroporphyrinogen decarboxylase is considered as their primary enzymatic defect, which might be inherited in most cases. Liver cell damage is an essential premise for the pathobiochemical genesis of chronic hepatic porphyrias, which can be regarded as "membrane disease". Alcohol and estrogens often lead to the clinical cutaneous manifestation of a chronic hepatic as "porphyria cutanea tarda".

The red cell enzymes and the metabolite excretion pattern were studied in 39 families with acute hepatic porphyrias, mainly acute intermittent porphyria, and in 19 families with chronic hepatic porphyria. From these studies it can be concluded that the both uroporphyrinogen synthase defect and the uroporphyrinogen decarboxylase defect seems to be transmitted by an autosomal dominant trait. Both enzyme defects in the erythropoietic system are not known to produce any deleterious effects.

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Immunologic involvement of kidney in obstructive jaundice

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Recent immunofluorescence (IF) studies have contributed to characterize glomerular lesions detected in some liver diseases (1, 2). Our previous observations have shown in man with obstructive jaundice glomerular changes by light and electron microscopy. Furthermore, glomerular deposits of immunoglobulin (Ig) and complement (C₃) have been demonstrated by IF procedure (3).

Among 15 patients with extrahepatic obstructive jaundice, 8 have shown mesangial and/or subendothelial fixation of anti-IgM serum; other Ig classes (IgG, IgA) have been detected only in 3 patients. Anti-C₃ and antifibrinogen serum fixation has been observed in 7 subjects.

On this basis, experiments have been carried out in rat after common bile duct ligation to further investigate the immunologic renal changes observed in man. Glomerular IF findings, systemic endotoxaemia, anti-endotoxins Ab, circulating immune complexes (Icx) and polymorphonuclear (PMN) phagocytosis are summarized in Tab. 1. IgG deposits have been early (3-5 days) detected in

TABLE 1
Obstructive jaundice in rat (common bile duct ligation)

Days	IF glomerular findings					Systemic endotoxaemia	anti-LPS Ab	PMN Icx	PMN phagocytosis
	IgG	IgA	IgM	C ₃	F				
Controls (n:9)	0	0	0	0	0	0	0	present	95%
3 days (n:5)	4/5	0	0	0	0	5/5	1/5	increased	40%
5 days (n:5)	4/5	0	0	2/5	0	3/5	2/5	increased	40%
7 days (n:5)	5/5	0	1/5	2/5	5/5	3/5	4/5	increased	38%
10 days (n:5)	5/5	2/5	3/5	3/5	4/5	3/5	5/5	increased	20%
15 days (n:5)	5/5	1/5	2/5	4/5	5/5	2/5	5/5	increased	15%

mesangium and subendothelial space. Later, the appearance of IgM, IgA and C₃ deposits has been observed together with an evident increase of IgG positivity in glomeruli. Systemic endotoxaemia, investigated by Limulus gelation test, has been early demonstrated in most of jaundice animals in concomitance to

the appearance and the progressive increase of anti-endotoxin Ab. Furthermore, circulating Icx have shown a significant rise while PMN phagocytosis ability appeared markedly reduced.

We can only speculate about the meaning of the described immunologic involvement of glomerulus. Firstly, the behaviour of Ig deposition suggests that the early detectable Ig deposit can be related to abnormal glomerular trapping of pre-existing circulating Icx, since RES clearance capacity is decrease as demonstrated in obstructive jaundice and other liver diseases. The significant decrease in PMN phagocytosis, observed in our experimental condition, seems in agreement with this statement.

The progressive increase in glomerular IgG deposits, observed in later stages of obstructive jaundice, also suggest that an immunologic activation can occur against exogenous antigens in presence of anatomic and functional liver damage.

The occurrence of systemic endotoxaemia and the presence of anti-endotoxin Ab, as observed in our experimental model, seems to suggest that a specific immunologic response to bacterial endotoxins, originated from the intestinal tract in jaundice condition, has been induced.

Anyway, the patho-physiologic role of the liver must be stressed since functional exclusion of the organ, as we have obtained in experimental hypertension and portocaval anastomosis, produces similar glomerular and humoral immunologic abnormalities.

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Functional study with ^{131}I Rose Bengal in chronic hepatitis and cirrhosis

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In order to evaluate the kinetics of ^{131}I tagged Rose Bengal (RB) in chronic active hepatitis we have determined in 11 patients the fractional transfer constants (f.t.c.) of the compartment model reported in Fig. 1a and, in comparison, in 11 patients with non-active cirrhosis. The model, whose parameters can be determined by the plasmatic curve only, has been widely used for many substances excreted by the liver: bilirubin (BRB), bromsulphophtaleine (BSP), Indiocyanin Green (ICG), Rose Bengal (RB). However, more complex models should be

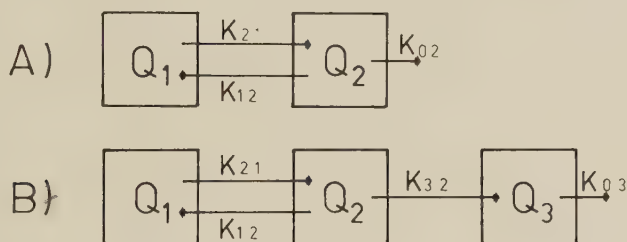


FIG. 1. A) Model of the kinetics of ^{131}I -Rose Bengal; B) Model proposed by Turco *et al.*

used for molecules undergoing conjugation by the hepatocyte—like BRB and BSP (Molino and Milanese, 1975; Berk *et al.* 1969; Anwer and Gronwall 1976). The bi-compartmental model may be considered reliable for substances (RB, ICG) excreted only by the liver, not metabolized, with no diffusion into extra-plasmatic spaces and no intestinal resorption. The addition of a third compartment (Fig. 1b), even if justified on theoretical basis (Turco *et al.* 1966) may impair the accuracy of the determined f.t.c.

The 22 patients were studied according to the A.I.S.F. criteria and the diagnosis was always confirmed by biopsy and laparoscopy. Table I reports the mean values of the f.t.c. and of K_e (fraction of the dye irreversibly removed from plasma in the unit time).

TABLE 1

"Mean and s.d. of ^{131}I -RB f.c.t."

	K_{21}	K_{12}	K_{02}	K_e
Chr. hepatitis	0.073 (± 0.013)	0.037 (± 0.018)	0.010 (± 0.005)	0.022 (± 0.009)
Cirrhosis	0.029 (± 0.016)	0.023 (± 0.015)	0.03 (± 0.03)	0.014 (± 0.008)
	$p < 0.005$	$p < 0.1$	$p < 0.1$	$p < 0.05$

The most significant differences between the two groups involve K_{21} and K_e . Data on normal subjects, cirrhosis and cholestatic jaundice are reported by Turco *et al.* (1968). Our findings are in agreement for cirrhosis. K_e and K_{21} (transfer constant from plasma to liver in the usual—somewhat arbitrary—identification of compartment 1 with the plasma and compartment 2 with the liver) are remarkably reduced. This impaired plasmatic clearance may mainly be due to the reduced liver blood flow, as shown by the proportional impairment of the disappearance rate of ^{198}Au -colloid in the same patients (cirrhosis: $\bar{m} = 0.089 \pm 0.012$; chronic hepatitis: $\bar{m} = 0.115 \pm 0.011$).

In chronic hepatitis K_{02} of RB is, on the whole, lowered and K_{12} increased. This would possibly mean an impaired transfer from the hepatocyte to the intrahepatic biliary tracts and an increased return from the cell to the plasma. K_{21} is also reduced (defective cellular uptake?). The modifications of the ratio $T^{1/2}_{\text{RB}}/T^{1/2}_{\text{Au}}$ has been already reported ($\bar{m} = 2.35$ in chr. hepatitis, 1.95 in cirrhosis).

Our data could be helpful for diagnostic purposes. By grouping the f.t.c. of RB in the discriminating function: $L = 18.18K_{21} + 0.27K_{12} - 1.85K_{02}$ ($p < 0.005$) we observed $\bar{L} = 1.32$ in chronic hepatitis and $\bar{L} = 0.48$ in cirrhosis;

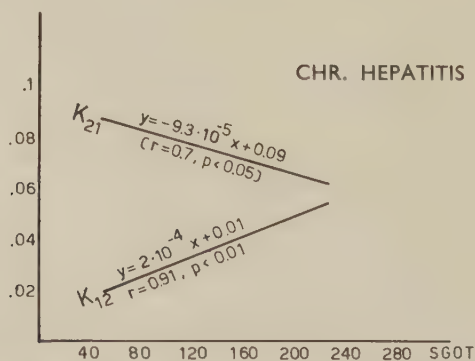


FIG. 2. Linear correlation between SGOT transaminase level and RB K_{21} and K_{12} in 11 cases of chronic active hepatitis.

discriminant level = 0.959 Discrimination into two groups according to this function was consistent with the histologic diagnosis in 21 cases out of 22, even in some cases where clinical and laboratory data were misleading.

The determination of the f.t.c. may also be useful to assess the degree of the hepatocitary damage. It is of some interest, from this point of view, to consider the linear relationships observed between the modification of K_{21} , K_{12} and the level of SGOT transaminases (Fig. 2) in chronic hepatitis.

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Glomerular function and increased renal tubular sodium reabsorption in hepatic cirrhosis

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To evaluate the amount and localization of increased renal tubular sodium reabsorption we investigated renal sodium excretion, clearance of sodium, distal sodium load, per cent of distal sodium reabsorption, and urinary sodium-potassium ratio during hypotonic saline diuresis (Chaimovitz *et al.* 1972) in 10 normal controls (Group I), and 64 cirrhotic patients classified into: 10 compensated cirrhotics (Group II), 39 decompensated cirrhotics (Group III), and 15 ascitic cirrhotics with functional renal failure (FRF) (Group IV). Renal plasma flow (RPF) and glomerular filtration rate (GFR) were examined under basal conditions. Results are shown in Table I.

RPF and GFR in Group II are similar to those observed in controls. Increased renal tubular sodium reabsorption was due to secondary hyperaldosteronism. Decompensated cirrhotics show a decreased RPF and GFR. Renal sodium reabsorption was greater than in Group II due to proximal and distal tubular sodium reabsorption. Impaired GFR might cooperate to diminished sodium excretion. Cirrhotic patients with ascites and FRF showed a marked decrease in RPF and GFR. The enormous increase in renal tubular sodium reabsorption is usually due to decreased GFR and enhanced proximal and distal tubular sodium reabsorption.

The second objective of our study was to achieve a therapeutic guide for rational treatment of decompensated cirrhosis. We divided 30 ascitic patients without FRF in two groups. Group A: 9 patients treated following the therapeutic guide of Summerskill and Baldus (1975). Group B: 21 patients treated as we recommended, once we knew the functional renal tests results. There were no statistical differences between both groups concerning clinical, laboratory data, or renal function tests. According to functional renal tests, each patient of Group B was treated with increasing doses of spironolactone or furosemide as the per cent of distal sodium reabsorption increased or as the distal sodium load decreased respectively. No major complications were observed with both therapeutic guides. Table II shows the differences between both groups.

TABLE 1
Renal handling of sodium in cirrhotic patients

	<i>RPF*</i>	<i>GFR</i>	<i>Na. exc.</i>	<i>CNa</i>	<i>CH₂O + CNa</i>	<i>CH₂/CH₂O + CNa</i>	<i>Na/K</i>
	<i>ml/mn/1,73 m²</i>	<i>μEq/mn/100ml</i>	<i>GFR</i>	<i>ml/mn/100ml</i>	<i>GFR</i>	<i>× 100 (%)</i>	
Group I (n = 10)	627.3 ±110.3	122.5 ±16.6	342.3 ±88.3	2.49 ±0.66	10.98 ±2.17	77.16 ±5.43	7.16 ±4.19
Group II (n = 10)	645.2 ±103.6	127.8 ±15.2	93.1 ±36.1	0.69 ±0.27	9.62 ±17.4	92.77 ±2.57	1.25 ±0.65
Group III (n = 39)	520.6 ±141.2	84.9 ±29.1	104.9 ±93.6	0.81 ±0.71	7.94 ±3.18	89.55 ±8.06	1.92 ±2.09
Group IV (n = 15)	215.9 ± 84.5	43.5 ± 3.2	45.3 ±37.1	0.36 ±0.29	4.90 ±4.40	92.38 ±3.77	0.53 ±0.35
PI-II	N.S.	N.S.	<0.001	<0.001	<N.S.	<0.001	<0.001
PI-III	<0.005	<0.01	<0.001	<0.001	<0.01	<0.001	<0.001
PI-IV	<0.001	<0.001	<0.001	<0.001	<0.001	<0.001	<0.001
PII-III	<0.02	<0.001	<N.S.	<N.S.	<N.S.	<N.S.	<N.S.
PII-IV	<0.01	<0.001	<0.01	<0.01	<0.01	<N.S.	<0.001
PIII-IV	<0.001	<0.01	<0.05	<0.05	<0.01	<N.S.	<0.02

* RPF = Renal Plasma Flow; GFR = Glomerular Filtration Rate; Na. exc. = Sodium excretion; CNa = Clearance of Sodium; CH₂O + CNa = Distal Sodium Load; CH₂O/CH₂O + CNa × 100 = Per cent of Sistol Sodium Reabsorption, Na/K = Sodium Potasium ratio.

TABLE 2
Differences between both therapeutic guides

	<i>Weight loose</i>	<i>Abdominal perimeter loose</i>	<i>Hospital Days</i>
	<i>Kg</i>	<i>cm</i>	
Group A (n = 9)	10.94 ±4.12	12.50 ±3.46	27 ±5
Group B (n = 21)	12.28 ±5.57	14.88 ±5.82	16.6 ±4.8
P	>0.3	>0.1	<0.001

We found no substantial differences concerning weight loose or abdominal perimeter loose; but there was a significant reduction in hospitalization days needed for compensation.

CONCLUSIONS

Functional renal tests allows us:

- To establish an initial prognosis.
- To choose the diuretics and doses that each ascitic cirrhotic is going to need.
- To shorten hospitalization.

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Urinary acidification in alcoholic cirrhosis: its association with hybrid type 1.2 renal tubular acidosis

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Cirrhosis of the liver has been shown to be complicated occasionally by renal tubular acidosis (RTA) of the distal type (classic RTA) (Shear *et al.* 1969). The mechanism by which liver insufficiency may interfere with tubular capacity to acidify the urine has remained unknown. It seemed reasonable to postulate that excessive tubular reabsorption of sodium reduced its delivery to the distal acidifying sites, thus diminishing the exchange of sodium for hydrogen ions. We investigated renal plasma flow (RPF), glomerular filtration rate (GFR), renal concentration, and maximal urinary acidification ability (L—arginine hydrochloride) in 8 patients with alcoholic cirrhosis, histologically proved, aged between 35 and 65 (mean 51), and 5 normal controls. Two patients could not lower their urinary pH during induced systemic acidosis for five consecutive days. No loss of proteins, phosphates, glucose, aminoacids, or lithiasis was demonstrated. No correlation was found with copper, seric globulins, basal sodium excretion, or with predisposition to hepatic encephalopathy. One patient showed incomplete RTA of the distal type. Natriuresis following furosemide administration partially corrected the defect in urinary acidification (Table 1) (Fig. 1).

This suggests that the rate of delivery of sodium to the distal exchange site may be a factor in tubular ability to generate steep urine: plasma hydrogen ion gradient (Better *et al.*, 1972). Another patient with hypostenuria and little reduction in GFR and RPF showed basal hyperchloremic metabolic acidosis and bicarbonaturia. Maintained acid load test provoked a reduction in plasma bicarbonate levels with excretion of low titrable acidity and ammoniuria. Bicarbonate overload showed a type 1, 2 hybrid RTA (Table 2) (Fig. 2).

Distal tubular acidosis complicating cirrhosis is widely known, but simultaneous involvement of proximal and distal tubular acidification defect has not been reported.

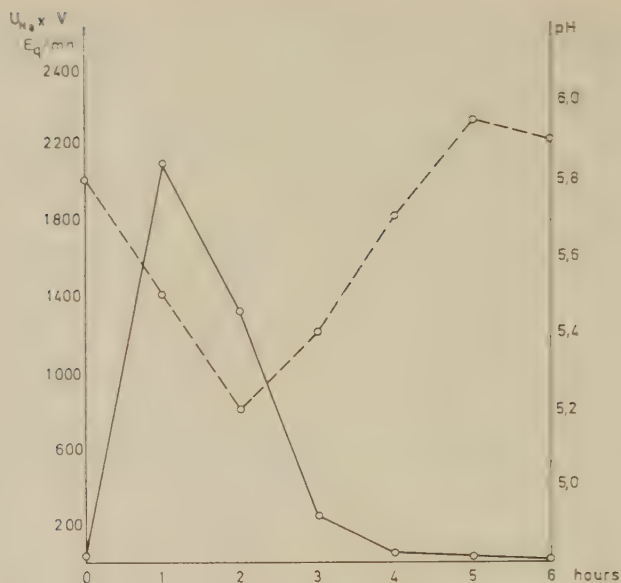


FIG. 1. Effect of furosemide administration (40 mg i.v.) on urine pH (— — —) and the rate of sodium excretion (—) during systemic acidosis induced by L-arginine hydrochloride. Prior to furosemide administration the urine was moderately alkalyne in the face of systemic acidosis.

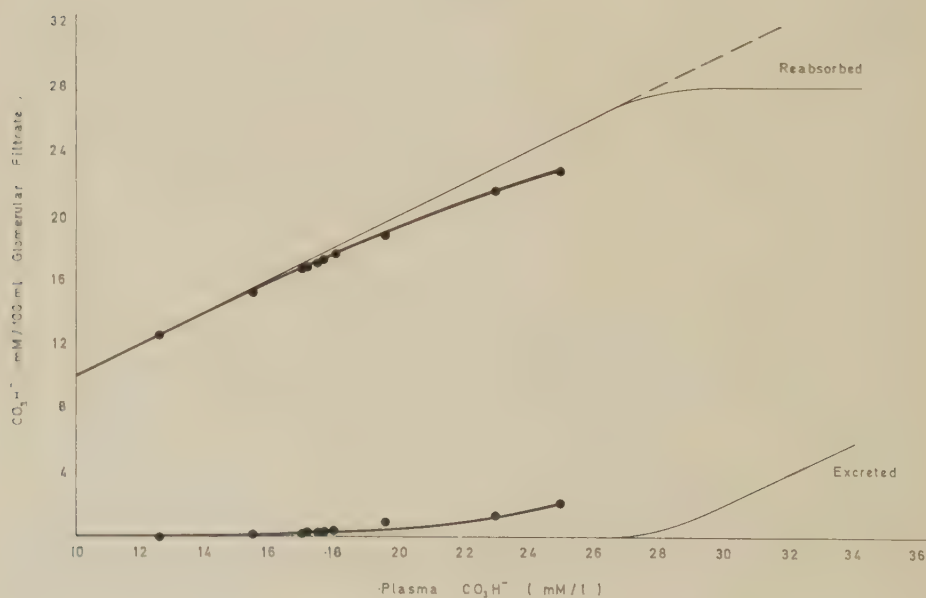


FIG. 2. Bicarbonate absorption and excretion in patient with type 1, 2 hybrid RTA.

TABLE 2

Urinary response to acid and alkali overload in patient with type 1.2 hybrid RTA

Day	Urine Sam- ple no.	V* ml/min	pH	T.A.		NH ₄ ⁺	CO ₃ H ⁻	H ⁻	NH ₄ ⁺ /H ⁻ / 100 (%)	Na		K	Cl	GFR ml/min/ 1.73 m ²	pH	pCO ₂	CO ₃ H ⁻	B.E.	Administered Substance
				μEq/ml/1.73 m ²	mEq/l					μEq/ml/1.73 m ²	mEq/l								
1	Basal	0.88	7.6	—	2.7	30.9	—	—	—	114	80	77	83.28	7.33	34.2	18	—3.3	↓ i.v. Arginine hydrochloride	
	4	2.60	7.3	2.65	4.9	58.5	—	—	—	169	262	447					—4.8		
	5	1.69	7.1	2.87	5.1	27.6	—	—	—	115	213	292		7.36	33.4	17.2			
	6	1.33	7.0	2.80	4.3	19.75	—	—	—	113	207	266							
	7	0.95	6.95	2.42	3.85	12.3	—	—	—	107	138	177							
2		0.47	5.5	12.7	7.7	—	20.40	37.75	—	26.8	44	42	72.12	7.25	28	12.6	—12.2	↓ Oral Arginine hydrochloride	
3	Basal	1.48	6.5	8.0	4.65	13.5	—	—	—	115	187	195	80.43	7.33	30	15.5	—7.6		
	1	2.08	6.6	9.2	6.65	21.4	—	—	—	56	41.6	49	81.57	7.38	30.2	17	—5	↓ Oral Sodium Bicarbonate	
	2	1.48	6.7	7.5	5.5	25.2	—	—	—	34	42	38	77.39	7.38	30.6	17.5	—5.1		
	3	1.12	6.8	4.5	3.14	25.8	—	—	—	57	45	43	68.26	7.38	32	17.7	—5.5		
	4	1.42	7.5	—	3.27	61.6	—	—	—	123	62.6	51	73.81	7.40	36	19.6	—3		
	5	3.91	7.5	—	9.0	117	—	—	—	183	74	47	56.64	7.47	39	23.0	—0.9		
	6	2.75	7.8	—	7.4	139	—	—	—	200	57	41	64.20	7.48	42	25.0	—1		

* V = minute volume; T.A. = Titrable acid; NH_4^+ = Ammonium excretion; CO_3H^- = Bicarbonate excretion; H^- = Net Hydrogen ion excretion ($\text{T.A.} + \text{NH}_4^+ - \text{CO}_3\text{H}^-$); GFR = Glomerular Filtration Rate (Cin); B.E. = Base excess.

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Functional renal failure in liver cirrhosis: the role of intrarenal shunts

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According to most authors (1-2), the nephropathy in liver cirrhosis is a functional and not an organic condition secondary to renal and intrarenal circulatory impairment. The pathogenesis of this alteration has not yet been clarified. Among the various hypothesis, the opening of intrarenal shunts has been considered a possible pathogenetic factor (3-4).

In order to verify this hypothesis intrarenal blood flow and intrarenal shunts were determined in 22 cirrhotic patients.

Mean renal blood flow (MBF), cortical blood flow (CBF) and juxtamedullary blood flow (JBF) were determined using the ^{133}Xe washout technique (5-6); in the same session intrarenal shunts were determined using radioactive microspheres.

25 μCi of human serumalbumin microspheres tagged with ^{131}I , (MISA- ^{131}I), 23-45 μ in diameter with a specific activity between 0.216-0.086 $\mu\text{Ci}/\text{mg}$, were suspended in 1 ml saline and injected slowly in the renal artery. After three minutes the same amount of tracer was injected in a periferal forearm vein. The radioactivity was registred over the lung for 6 minutes. The intrarenal shunts were calculated according to the formula $S = \frac{\text{RA}-\text{BG}}{\text{PV}-\text{RA}}$.

The increase in counts per minute after injection in the renal artery (RA-BG) divided by the counts per minute increase after injection in the periferal vein (PV-RA), that is the 100% reference, is a function of the amount of blood that shunts the renal capillary net-work.

The normal range for the renal and intrarenal blood flow was determined in 12 controls, and for the intrarenal shunts in 6.

In our patients with liver cirrhosis a decrease of MBF was found ($\text{MBF} = 2,09 \pm 0,63 \text{ ml/g/min}$; n.v. = $3,15 \pm 0,55 \text{ ml/g/min}$).

Since post-mortem studies of the kidneys in patients with liver cirrhosis have never shown changes in the volume of the kidneys, it is very likely that the decrease of MBF found by the Xenon method reflects a decrease of total renal blood flow.

As for the intrarenal circulation in agreement with the literature, we found a redistribution of intrarenal blood flow consisting in a decrease of cortical perfusion ($\text{CBF} = 2.84 \pm 0.68 \text{ ml/g/min}$; n.v. = $4.49 \pm 1.09 \text{ ml/g/min}$) and maintenance of a normal juxtamedullary blood flow ($\text{JBF} = 0.51 \pm 0.17 \text{ ml/g/min}$; n.v. = $0.68 \pm 0.16 \text{ ml/g/min}$).

The decrease in renal blood flow was also found in patients that did not show any clinical or humoral signs of nephropathy and it was not correlated with either the aetiology of the cirrhosis, nor with the severity of the liver disease as evaluated by biochemical parameters nor with the degree of portal hypertension.

In the 22 patients with liver cirrhosis in which intrarenal shunts were studied they were found to be higher than in the controls (shunts = 5.5 ± 2.8 ; n.v. = 1.9 ± 0.5).

Moreover we found a significant negative correlation between intrarenal percentage of shunts and MBF and CBF ($r = -0.54$, $p 0.01$ and $r = -0.64$, $p 0.001$).

Therefore it is likely that intrarenal shunts are responsible, at least partially, for the decrease of MBF and redistribution of intrarenal blood flow.

The pathogenesis of these shunts is poorly understood. Opening of shunts seems independent of the severity of liver disease as evaluated by biochemical parameters and of portal hypertension degree.

This conclusion is suggested by the lack of correlation between intrarenal shunts and liver function tests and wedged hepatic venous pressure found also in our cases.

As for intrarenal localization of shunts since they were found to be correlated to CBF, it is likely that they are situated in the renal cortex.

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Acute hepatic intoxication in rats by D-Galactosamine. Protective effect of D-Penicillamine

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D-Galactosamine (G) intoxication has been used as a model for the acquirement of basic knowledge on metabolic functions of the hepatic cell, studying the alterations induced by its administration.

A double-blinded study in 64 rats type Wistar and female, divided in four groups, was performed. Group I, with 20 elements acted as control. Group II, with 20 animals received a single dose (375 mg/kg) intraperitoneal of G. Group III and IV, with 12 rats each, were protected by means of 50 and 100 mg/kg i.p. of D-Penicillamine (P) for a time of 21 days prior to the administration of the G dose. In all cases, sodic penthobarbital, adecuated for i.p. administration, was used as anesthetic.

An optic and electronic microscopy morphologic study was performed in all the groups. It was clear, for O.M., that rats receiving only G presented characteristic changes consisting in unicellular or focal necrosis with scarce periporthal infiltration, acidophilic bodies and lipidic degeneration. Reticulin was well conserved. Prevention with P prevents periportal infiltration and focal necrosis and does not lipidic degeneration.

Electronic microscopy called attention to a great proliferation of smooth endoplasmic reticulum and reduction of rough endoplasmic reticulum, fatty infiltration, autophagic vacuoles, nucleolar fragmentation and diverse mithochondrial changes and the frequent presence of liposomes. Penicillamine averts rough endoplasmic reticulum lesions imputed to galactosamine and the rest of cellular organells showed an injury similar in type, but much less in degree.

At the same time, the next biochemical determinations were carried in serum: SGOT, SGPT, alkaline phosphatase, total bilirubin, glucose, urea, cholesterol, uric acid and total protein level. A significative correlation between SGOT, SGPT and glucose was observed among II-III, II-IV and III-IV groups, sug-

gesting a positive protector effect of penicillamine for the group of 100 mg/kg dose, as assessed by the pathologic study.

Uridilate pool in the hepatic cell followed a parallel variation to the galactosaminic lesions, as already stated by other authors.

Protective effect observed by D-Penicillamine, might be related to the depletion of some heavy metals, principally copper and zinc, that actuate as cofactors in different enzymatic stages at the hepatic cell, interfering the UDP-hexosamine formation and subsequent uridilate captation.

Relationship between plasma availability of prednisolone after therapeutic dose of the drug and side effect appearance

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Prolonged corticosteroid therapy may produce serious undesirable side effects. Various mechanisms were suggested to be involved in determining susceptibility to adverse reaction to steroids, such as impaired liver function (1), low level of albumin (2, 3), with higher percentage of unbound prednisolone, altered conversion of prednisone to prednisolone (observed by some, 4, 5; but not confirmed by others, 6-9).

We have recently observed that plasma availability of prednisolone after a therapeutic dose of the drug showed wide reproducible individual variations both in normal and in patients affected by chronic active liver disease. We wondered whether patients with higher level of plasmatic prednisolone more easily presented side effect appearance after prolonged corticosteroid therapy than patients with lower level.

In order to clarify this point, the relationship between prednisolone availability, albumin level and side effects was evaluated, in patients treated with 20 mg of prednisolone for at least 1 year.

Case material 12 out of 25 patients, in which plasma prednisolone availability had been determined (9), had been treated with 20 mg of prednisolone, daily, as a maintenance dose. After at least 1 year of therapy, the appearance of side effects was evaluated. The descriptions of the casistics and the methods employed, are reported in the previous paper (9).

RESULT AND DISCUSSION

No relationship between albumin level and side effect appearance was observed (Fig. 1). This result is not in accordance with those obtained by Lewis *et al.*, (2) and Uribe *et al.*, (3) which revealed a correlation between the frequency of steroid side effects and the serum albumin. However, the casistics are not

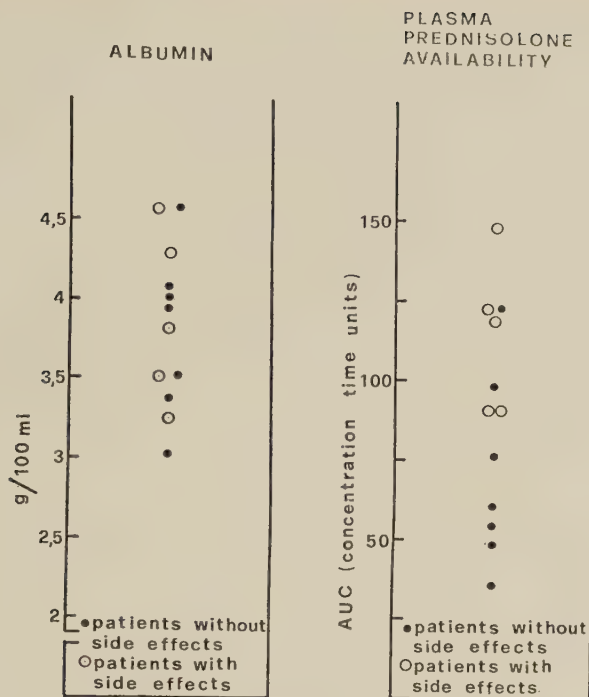


FIG. 1. Relationship between side effects appearance, albumin level and plasma prednisolone availability.

comparable, since none of our patients had albumin level inferior to 3 g% ml, while in the subjects reported by the above mentioned authors, serum albumin concentration was often less than this value.

On the contrary, side effects were more easily observed among the patients with major values of prednisolone availability (Fig. 1). The observed adverse reactions included the following: osteoporosis (2 patients), diabetes (1 patient), moon facies (1 patient), duodenal ulcer and moon facies (1 patient).

The interindividual differences in plasma availability, after a similar dose of prednisolone could explain that a desirable result may occur in certain subjects, toxicity in others and no effect whatsoever in still others. In fact, more availability of the drug, more frequent the side effect appearance.

Therefore, we believe that the estimation of the plasma availability of prednisolone after a therapeutic dose, could be considered as being of value in individualizing the correct dosage, so as to avoid side effects and perhaps to control the disease itself.

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Use of gray-scale ultrasound-tomography in demonstrating liver metastatic diseases

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The sonographic pattern of liver metastatic lesions depends on the distribution of the metastases in the liver itself. Solitary metastases, focal metastatic lesions and multiple metastases may occur. Schematically, the following sonographic patterns of liver metastases can be recognized: sonogenic nodules, transsonic nodules, surrounded by an echoconstant zone, and sonolucent area in a sonogenic parenchyme.

In discussing the sonographic pattern of liver metastases, the sonographic aspect of the metastases is a very important factor: a homogeneous or a heterogeneous distribution of echoes themselves may occur.

The purpose of this paper is to show that there is a definite sonographic pattern in liver metastatic diseases. A strong correlation exists between the sonographic liver pattern and the etiology of metastatic lesions derived from the gastro-intestinal tract.

In total 38 cases of histologically proven liver metastases were studied. Of these metastases 21 were solitary, 4 were focal and 13 had a multiple distribution. There were 22 with a heterogeneous sonographic aspect and 16 with a homogeneous aspect.

Most multiple metastases are heterogeneous; few are homogeneous, and these are exceptions such as metastases from adenocarcinoma of the stomach, which are nearly always heterogeneous. The same is true for the solitary metastases: only stomach adenocarcinoma have constantly a homogeneous sonographic aspect. All focal metastases are heterogeneous in echoes distribution. Finally, the sonographic patterns were as follows: 12 cases were sonogenic nodules; 12 occurred as transonic areas, surrounded by a sonogenic rim and 14 cases were sonolucent. In the series of the solitary metastases the sonogenic nodule predominates. A complete homogeneous distribution of echoes occurs here with clearcut outlines of the nodule and a marked contrast between the echodense center and the echolucent surrounding zone. The appearance of a transonic area within a normal liverparenchyme, described by Taylor (4) as being characteristic of liver metastases, was found in only one patient in the series of liver metastases derived from the gastro-intestinal tract. However this

picture was dominant in the miscellaneous group. The contrast between the sonolucent centre and the echo-dense surrounding zone is even more pronounced in these fast growing solitary metastases of the miscellaneous group. This is in agreement with Carlsen's report (1) that small metastatic lesions are likely to be less echogenic and as these lesions progress, there is an internal echoes display from degeneration or necrosis.

The sonographic pattern of focal metastatic lesions appeared as "compact clusters of medium sized echoes". A clear contrast is visible between the so called echolucent zones and the centrally located dark echoes: this picture has been labelled "bull eyes".

Multiple, large or conglomerate metastatic lesions produce on the ultrasonic scanning only a change in the pattern of internal echoes in the liver. They are easy to recognize because of their heterogeneous character. This may be a reason why multiple metastatic lesions are easiest to detect and were originally described by ultrasonic scanning. In this small number of cases, the homogeneous distribution of the echoes is usually typical for adenocarcinoma of the stomach. A heterogeneous sonographic aspect is predominant in colon carcinoma, but also liver metastatic lesions derived from carcinoma of the thyroid gland, from an osteosarcoma, present a heterogeneous character (3).

Although based on an admittedly small number of cases of liver metastases, it can be concluded that a sonographic distribution consisting of many sonolucent zones in which centrally echoes occur, is typical of adenocarcinoma. A homogeneous distribution of echoes is typical for adenocarcinoma of the stomach. Most frequently encountered sonographic patterns are sonogenic nodules and sonolucent areas: the former as solitary, the latter as focal or multiple metastatic lesions.

In demonstrating liver metastatic lesions, the gray-scale ultrasound tomography is an easy and accurate method, but the only disadvantage of the ultrasound technique is that "in abdominal work involvement of a doctor is necessary!" (2).

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Gamma-glutamyltranspeptidase in patients with Dubin-Johnson syndrome

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Congenital hyperbilirubinemia of the Dubin-Johnson type is considered to be in disorder of the excretory liver function, leading to the accumulation of pigment in otherwise normal hepatocytes (Dubin, I.N., 1958, Arias, I.M., *et al.*, 1964) and increase of direct bilirubin without affecting the bile acid metabolism.

The most sensitive liver function test with diagnostic significance in this type of hyperbilirubinemia is the sulfobromophthalein test which is excreted with delay into bile, causing a reduced transport maximum, but retaining a normal storage capacity (Shani, M., *et al.*, 1974).

In a previous study, (Merdler, C., *et al.*, 1976) administration of phenobarbital in patients afflicted by this variety of hyperbilirubinemia, revealed improvement of hepatic function including reduction of serum bilirubin level, and enhanced clearance of sulfobromophthalein (BSP).

Gamma—glutamyltranspeptidase (GGTP) is an enzyme whose level is elevated in various hepatobiliary conditions. It is not included in conventional hepatic enzyme tests, all of which are normal in patients with Dubin-Johnson syndrome.

Elevation of this serum level was considered at first to be indicator of chronic hepatitis (Szczeklik, E.M., *et al.*, 1961), but it was also applied as a screening enzyme for other disorders of the liver and bile systems. Elevated GGTP level can be used to detect mild changes in liver function, as in apparently normal blood donors who denied a history of hepatitis. In the course of acute hepatitis, no prognostic value has been found in GGTP follow-up, although it is usually the last enzyme to return to normal (Dragosics, B., *et al.*, 1976). An isolated increase of GGTP may be the first sign of cholestasis or may suggest intake of drugs (Rosalski, S.B., *et al.*, 1971) or alcohol (Zein, M., 1970).

In order to determine the diagnostic value of this enzyme in congenital hyperbilirubinemia of Dubin-Johnson syndrome, the serum level was determined in 10 patients affected by this form of jaundice.

METHODS

10 ambulatory patients diagnosed as suffering from Dubin-Johnson syndrome were included in the study. There were 6 males and 4 females, the ages ranging from 14 to 60 years (average 32 years).

The diagnosis had been previously made, on the basis of liver biopsy demonstrating a dark pigment in otherwise normal liver cells (Dubin, I.N., 1958), a positive family history, intermittent or persistent hyperbilirubinemia, prolonged BSP retention, normal relative storage capacity, a reduced transport maximal rate and impaired visualization of the gallbladder on oral cholecystography.

The subjects were in good health, apart from clinical jaundice. All medications were withheld two weeks before the study.

STUDIES

Blood samples were obtained from all patients after overnight fast and serum total and direct reacting bilirubin, glutamic oxalacetic transaminase, alkaline phosphatase and cholesterol were estimated.

The serum level of GGTP was estimated using glutamyl—p nitroanilide as substrate (Szasz, G., 1969; Albert, Z., *et al.*, 1964). The normal range is 6-28 I.U./liter for males and 4-18 I.U./liter for females (Albert, A., *et al.*, 1964).

In one patient (male, 34 years) BSP retention test at 45 and 120 minutes was determined after intravenous administration of a 5 mg/kg body weight dose, before and after two week treatment with phenobarbital 0.1 g t.i.d.

RESULTS

The total and direct bilirubin levels in the patients are shown in Table 1. The average total serum bilirubin was 2.84 ± 0.67 mg/100 ml. Average level of direct bilirubin was 1.77 ± 0.43 mg/100 ml.

Table 2 shows the level of GGTP in the same patients (patients 1-6 are males, 7-10 are females). Among all patients normal values of enzymatic activity were found.

In one case (Patient 5), BSP retention test revealed 7% and 10% retention at 45 and 120 minutes respectively. After administration of phenobarbital, BSP retention decreased to 4% at 45 minutes and 7% at 120 minutes. At the same time, GGTP level rose from 81.U./liter to 25 I.U./liter.

Normal values of glutamic oxalacetic transaminase, alkaline phosphatase and cholesterol were obtained in all patients.

TABLE 1

Total and direct bilirubin levels in sera of Dubin-Johnson patients

<i>Patient</i>	<i>Serum bilirubin, mg/100 ml</i>	
	<i>Total</i>	<i>Direct</i>
1	2.5	1.5
2	3.1	1.8
3	1.9	1.1
4	2.2	1.6
5	4.3	2.8
6	2.6	1.4
7	3.5	1.9
8	2.7	1.8
9	3.2	2.1
10	2.4	1.7
Mean \pm SD	2.84 \pm 0.67	1.77 \pm 0.43

TABLE 2

Serum GGTP level in Dubin-Johnson patients

<i>Patient</i>	<i>GGTP level (I.U./liter)</i>
1	7
2	9
3	13
4	26
5	28
6	17
7	10
8	13
9	8
10	9
Mean \pm SD	12 \pm 5.5

DISCUSSION

Gamma-glutamyltranspeptidase has proved to be a very sensitive screening enzyme for several hepatobiliary disorders, detecting mild disturbances in both hepatic function and biliary excretion. High concentrations of this enzyme are normally found in pancreatic cells, hepatocytes, small bile ducts, small intestinal epithelium and renal tubular cells (Albert, Z., *et al.*, 1969).

In the present study, the correlation of the presumed enzymatic defect of Dubin-Johnson syndrome with the GGTP activity was examined.

There does not appear to be a direct correlation between the GGTP level and the serum bilirubin in these patients.

Apparently, in the Dubin-Johnson syndrome, the biliary excretion of GGTP follows a similar pathway to that of alkaline phosphatase and bile salts, unlike bilirubin, iodopanoic acid and BSP, which are retained to a certain extent.

GGTP is a valuable parameter in the classification and characterization of various liver disorders. In Dubin-Johnson syndrome further investigation is warranted in two directions: (1) histochemical determination of enzyme activity within hepatocytes, (2) the correlation with BSP retention during phenobarbital administration which we have previously shown promotes hepatic clearance of BSP.

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Mechanism of kidney involvement in the course of chronic hepatitis

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To assess the role of HBsAg immune complexes (IC) in the pathogenesis of glomerulonephritis (GN) (Combes *et al.*, 1971, Brzosko *et al.*, 1974, Hirschel *et al.*, 1977), we investigated 23 patients with GN and hepatitis who were selected from a population of 130 consecutive patients with histologically proven GN. Eleven patients (Group I) had HBsAg positive chronic hepatitis, 4 (Group II) essential mixex cryoglobulinemia (EMC) and 8 (Group III) HBsAg negative hepatitis. The prevalence of HBsAg positive hepatitis (8.4%) in this population is not significantly different from that of HBsAg negative hepatitis (6.1%). The morphological pattern of GN in both Group I and Group III encompassed a wide variety of glomerular lesions ranging from membranoproliferative GN (MPGN) to minimal glomerular lesions, while all the four patients with EMC had MPGN. Similarly to Group III, none of the patients within Group I had glomerular deposits of HBsAg as shown by direct immunofluorescence (IF) negative staining of frozen kidney sections. These observations suggest that the association between the serum HBsAg and GN occur by chance, rather than by a causal relationship. Glomerular deposits of HBsAg were neither found in the three patients with MPGN and positive staining for Ig's and complement. On the other hand, three out of four patients with EMC and MPGN had heavy deposits of HBsAg as shown by direct IF staining. These patients had also anti-HBs antibodies in their serum. The deposits of HBsAg were observed in the glomeruli, around the tubuli and within the small arteries and were associated with deposits of immunoglobulins G, A, M, and complement components of both the classic and alternate pathways. Similar deposits were observed within the Kupffer cells of the liver in one patient with EMC. The kidney of these patients contained also deposits of Rheumatoid factor (RF) as shown by the positive staining with fluoresceinated aggregated IgG and its blocking by pre-treatment with unfluoresceinated anti-IgM serum (Rossen *et al.*, 1975). This finding shows that the IgM deposits within the kidney are endowed with anti-IgG or rheumatoid activity and provide the demonstration that a complex IgG-

IgM (k), identical to that of serum, is deposited within the kidney. To solve the problem whether the HBsAg positive immunofluorescent staining results from the binding of the Fc portion of the fluoresceinated anti-Hbs molecule to the deposited RF, or to a specific reaction with the virus surface antigen, two tests were set up: a) pretreatment of tissue sections with the unfluoresceinated anti-IgM serum to block the deposited RF, followed by direct immunofluorescent test for HBsAg; b) treatment of the tissue sections with the Fab portion of the fluoresceinated rabbit anti-HBs. Both tests provided negative results indicating that the previous immunofluorescent positive reaction for HBsAg in kidney and liver sections was unspecific, i.e. an artifact due to the RF activity of the IgM deposits.

In conclusion the kidney involvement in HBsAg chronic hepatitis encompasses a wide variety of morphological types of GN and is not associated to HBsAg IC in glomeruli. The positive immunofluorescent staining for HBsAg in the kidney of patients with EMC is an artifact due to the binding of the Fc portion of the fluoresceinated molecules to the deposited RF. An IgM (RF)—IgG complex is found in the kidney of patients with EMC and may well be responsible for the tissue injury. The role of HBsAg in the genesis of GN remains unproved. Whenever deposits of RF occur in the tissues, the fluoresceinated Fab molecules instead of the whole antibody molecule should be used for the demonstration of specific antigen.

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Different production and utilization of 16α -hydroxylated steroids in feminized and non feminized cirrhotic patients

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The 24 hours urinary excretion of 11 deoxy-17 ketosteroids (Androsterone, Etiocholanolone and Dehydroepiandrosterone = 11-DO-17KS) 16α hydroxi-Dehydroepiandrosterone (16α OH-DHA) and Androstentriol (A_3) has been measured in male patients with liver cirrhosis assesed by biopsy with or without clinical signs of feminization (Gynecomastia of more than 10 cm in diameter and testicular atrophy with less than 3cm in longitudinal diameter). Measurements have been carried out under basal condition, and after 100mg Dehydroepiandrosterone-Sulfate (DHA-S) intravenous infusion to test possible differences in steroid metabolism between controls and both cirrhotic groups. The infusion has been performed over 4 hours, starting between 9-10 am, and blood samples have been taken, before and at one hour intervals during the DHA-S administration. Blood was immediately centrifugated and plasma used for estrogen determination.

10 male cirrhotic patients have been studied (5 with and 5 without clinical signs of feminization) and results compared with a control group of 5 male subjets of the same age. The measurement of 11-DO-17KS, 16α OH-DHA and A_3 has been carried out by Gas Liquid Chromatography using two different columns OV-1 and OV-225 as published elsewhere (1). Plasma estrogen concentrations have been determined by Radioimmunoassay using an antibody with high specifity against estradiol (less than 1% cross reactivity with estrone and estriol) and no further purification (2).

RESULTS

Basal 24 hours urinary excretion of 11-DO-17KS, 16α -OH-DHA and A_3 are expressed in table I. Patients with liver cirrhosis show a reduced 11-do-17KS excretion related to controls ($p < 0.02$) specially the feminized group.

Urinary 16α -OH-DHA is enhanced in both cirrhotic groups ($p < 0.05$) and A_3 excretion in the non feminized patients ($p < 0.05$).

TABLE 1

Basal urinary excretion ($\mu\text{g/d}$)

		11DO- 17KS	16-OH DHA	A ₃
Controls	x	1746	124	338
	sem	450	48	256
Non feminized cirrhotics	x	1075	210	450
	sem	286	117	188
Feminized cirrhotics	x	786	246	284
	sem	225	91	79

Under basal conditions, plasma estrogen levels are found to be higher in cirrhotic patients than in controls ($p < 0.025$) with higher levels in the feminized group, as shown in Table 2.

TABLE 2

Post-DHA-S urinary excretion ($\mu\text{g/d}$)

		11-DO 17-KS	16-OH DHA	A ₃
Controls	x	8705	1717	1237
	sem	1330	293	645
Non feminized cirrhotics	x	2763	6139	2223
	sem	666	2511	760
Feminized cirrhotics	x	2150	3885	1611
	sem	657	1511	411

After DHA-S administration (Table 2) the differences between cirrhotics and controls are more evident. Feminized patients show a more significant reduction of 11-d0-17KS excretion than non feminized ($p < 0.005$) and those are significantly lower ($p < 0.01$) than controls.

A₃ and 16 α -OH-DHA together are enhanced in all cirrhotics being the increase more evident in the non feminized group ($p < 0.005$).

The differences of plasma estrogen levels during DHA-S infusion between non feminized cirrhotics and controls are only significant during the first hour,

whereas in the feminized group are maintained over the whole infusion period (4 hours). Table 3 ($p < 0.025$).

TABLE 3

Plasma estrogen concentrations (pg/ml)

		<i>Basal</i>	<i>DHA-S infusion</i>			
			<i>1 h.</i>	<i>2 h.</i>	<i>3 h.</i>	<i>4 h.</i>
Controls	x	53	54	54	50	53
	sem	3	2	5	6	8
Non feminized cirrhotics	x	66	63	59	64	60
	sem	5	7	8	15	11
Feminized cirrhotics	x	77	84	86	76	76
	sem	8	5	12	8	10

DISCUSSION

Results obtained in this experiment seem to indicate not only differences in the metabolic management of the DHA-S substrate between cirrhotic patients and control subjects, but also between feminized and non feminized cirrhotics.

The reduced excretion of 11-DO-17KS in liver cirrhosis described earlier by Sonka (3) is specially evident in the feminized group both basally and after DHA-S administration. There is on the other hand a higher excretion of 16α hydroxylated compounds ($A_3 + 16\alpha$ -OH-DHA) in cirrhotics (1) indicating an enhancement of 16α hydroxylating capability in the diseased liver. That greatest amounts are present in the non feminized cirrhotics, could perhaps indicate a better utilization of 16α hydroxylated steroids as precursors for estrogen formation in the feminized as shown before in hepatoma and breast cancer (4, 5) and as suggested by the enhanced plasma estrogen levels in those patients. A positive correlation between clinical sign of feminization and estrogen levels has been also observed.

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Autoantibodies to collagen in chronic liver diseases

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In the search for specific disease markers we became interested to investigate, whether anticollagen antibodies are present in patients with chronic liver diseases. Increase in collagen synthesis and degradation, as seen in chronic liver diseases especially of alcoholic origin, might lead to the formation of immunogenic collagen breakdown products and thus to the induction of autoantibodies to collagen. Other diseases characterized by chronic inflammation and proliferation of connective tissue were shown to be associated with the appearance of circulating anticollagen-antibodies (ACA). We used second antibody technic in our radioimmunoassay for ACA. The antigen was tritium-labeled by acetylation, granting the uniform labeling of the whole collagen molecule. Three groups of patients with liver diseases were compared as to the binding of radiolabeled collagen with a group of 24 normal persons: acute hepatitis, chronic hepatitis, chronic alcoholic hepatitis. The mean dpm-value of the control group plus twofold standard deviation was taken as significance limit for positive results. Among the patients with acute hepatitis (16) only two had IgG-anticollagen antibodies, while all were negative for IgA-ACA or IgM-ACA. In contrast, ACA could be demonstrated in 17 of 27 patients with chronic alcoholic liver disease and in 12 of 19 patients with chronic non-alcoholic liver disease. By using group-specific second antibodies we found that most of the ACA in chronic liver disease were of the IgA antibody class. This is in contrast to the situation in rheumatoid arthritis, where 90 per cent of ACA belong to the IgG immunoglobulin class. Our present study was performed with type I collagen, since this collagen can be extracted from human tissue without pepsin digestion. Future studies must center on the demonstration of antibodies to type III collagen, since this collagen seems to be a better indicator of active fibrosis and collagen turnover.

A probabilistic evaluation of nuclear liver imaging

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An attempt to determine the value of nuclear liver imaging in the clinical decision-making process was made by analyzing 4,886 histologically documented cases published in the literature between 1963-1976. The sensitivity of the test was found to be 0.81 and the specificity 0.85. These values are similar to those published in the literature.

A new parameter, the "Gain in Probability" $\Delta P(\%)$ was defined such as: $\Delta P(\%) = \text{Post-Scan Probability} - \text{Apriori Probability}$, Whereas the Post-Scan Probability = $P(D+ / T+)$ for positive scans correctly predicting liver disease and $P(D- / T-)$ predicting correctly no liver pathology, according to Bayem's Theorem. The Apriori Probability was considered as the incidence of disease $P(D+)$ or non-disease $P(D-)$ for positive and negative scans accordingly.

The "Gain in Probability" $\Delta P(\%)$ was found to be inversely proportional to $P(D+)$ in the case of positive scans. This correlation was found to be linear: $y = -0.60x + 0.61$, $r = 0.99$. The "Gain in Probability" was found to be directly proportional to $P(D-)$ in the range of $P(D-) = 0.46$ to 0.99 : $y = 0.39x - 0.34$, $r = 0.99$. When $P(D-)$ was lower than 0.46 the function was that of a power curve with a maximum at $P(D-) = 0.30$ and the equation was: $y = 0.54x^{0.56}$, $r = 0.94$.

The overall "Gain in Probability" $\Delta P(\%)$ was 0.27 and 0.40 for positive and negative scans, respectively. In "focal liver disease" i.e. primary and secondary neoplasms, abscesses, cysts etc. the $\Delta P(\%)$ was higher 0.50 and 0.27 for positive and negative scans. In the case of diffuse parenchymal liver diseases $\Delta P(\%)$ was 0.82 for positive scans and 0.27 for negative scans.

From the function of $\Delta P(\%)$, vs. $P(D+)$ and $P(D-)$ predicted values of $\Delta P(\%)$ can be calculated and compared to those found by direct calculation as described earlier. Values higher than predicted were found in malignancies of the G.I. tract whereas in carcinoma of the lung the calculated value was lower than the predicted value.

It seems to us that this type of analysis could be of value in many other clinical tests that can be validated objectively.

Clinical and pathophysiological considerations on the acid-base balance in cirrhosis of the liver

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BACKGROUND

Exploration of the acid-base balance of patients with cirrhosis of the liver has shown that by far the more frequent disorders are respiratory alkalosis in the milder cases and metabolic acidosis in the more severe cases.

The hyperventilation of patients with respiratory alkalosis is attributed to hyperammonemia, hypoxemia, and reduction of the ventilation/perfusion ratio; and according to widespread opinion, metabolic acidosis reflects an increase of lactate and pyruvate content in the blood.

CLINICAL CASES AND RESULTS

Our study involved a total of 58 patients, mean age 55.3 years; of these, 47 were male (81%), and 11 female (19%).

The reason for hospitalization was a hemorrhagic complication (hematemesis and/or melena) in 26 cases (44.8%); the onset of ascites in 30 cases (51.7%); and extant hepatic coma in 2 cases (3.4%). Table 1 lists the anatomical variety

TABLE 1

Acid-base balance situation in 58 cases of cirrhosis of the liver. Summary of clinical cases
Anatomoclinical type of cirrhosis of the liver

<i>Acid-base situation</i>	<i>Postnecrotic</i>	<i>Alcoholic</i>	<i>Biliary</i>	<i>Totals</i>
Respiratory alkalosis				
plus metabolic acidosis	9	20	1	30 51.7%
Respiratory alkalosis	4	10		14 24.1%
Normal	1	5	1	7 1.2%
Metabolis acidosis		4		5 0.6%
Metabolic alkalosis	1	1		2 0.3%
Respiratory acidosis		1		1 0.17%
TOTALS	15	41		58

of cirrhosis and the acid-base situation for each case. As can be seen, the more numerous groups are contributed by patients with pure respiratory alkalosis (14 cases) and those with metabolic acidosis (30 cases).

DISCUSSION

According to the literature, the most common acid-base disorder associated with cirrhosis of the liver is respiratory alkalosis, with metabolic acidosis occurring only in the more severe cases. Also in our experience the less severe cases showed respiratory alkalosis and the more severe ones showed a metabolic acidosis definitely of the lactic acid type. Still, the more numerous (30 cases) and also the more interesting group was that of patients with combined respiratory alkalosis and metabolic acidosis. In terms of clinical severity these patients occupy a half-way position between those with "pure" alkalosis and those with "pure" acidosis. It seems to follow that the status of the acid-base balance provides a fairly faithful reflection of the clinical stage of cirrhosis.

At this point we tried to determine whether or not the metabolic acidosis component of these 30 patients was of the lactic acid type. Since in these patients the PaCO_2 values and blood lactic acid assays were very much the same as in patients with pure respiratory alkalosis (to the point where a PaCO_2 /lactate regression line could be constructed for each of the two groups, as shown in Fig. 1), we concluded that in both groups the hyperlactacidemia was a consequence of hypocapnia and could not therefore be the cause of metabolic acidosis.

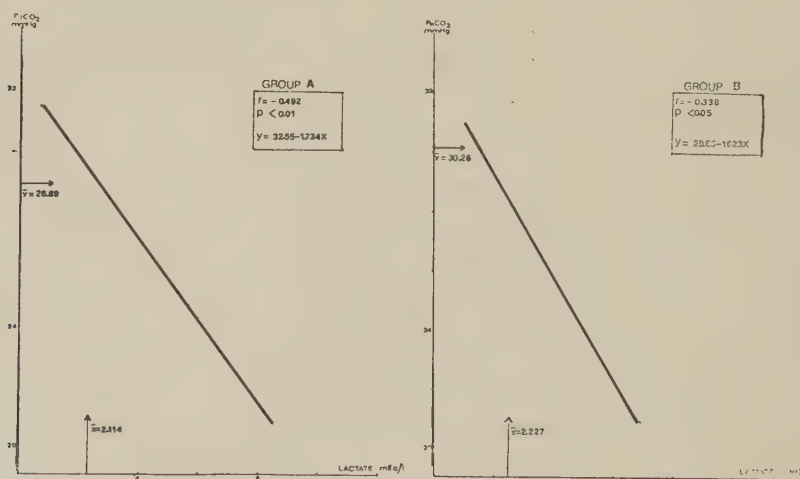


FIG. 1. Regression straight lines for comparison of lactate versus PaCO_2 in 44 cases of cirrhosis of the liver. Group A: Respiratory alkalosis with metabolic acidosis (30 cases); Group B: Respiratory alkalosis (14 cases).

Said cause must be sought elsewhere—possibly in the difficulty with which the cirrhotic organism accomplishes the renal excretion of fixed acid radicals originating in normal metabolism or produced in excessive amounts in the presence of cirrhosis.

SUMMARY

The study of the acid-base balance of 58 patients with cirrhosis of the liver suggests a possible relationship between the stage of evolution of the disease and the associated acid-base unbalance. Usually the milder, clinically compensated cases show a situation of respiratory alkalosis; those going through a stage of aggravation with complicating events (hematemesis and/or melena, ascites, portosystemic encephalopathy) show a combination of respiratory alkalosis and metabolic acidosis; and the more severe cases show pure metabolic acidosis.

In this last group the acidosis is of the lactic acid type and can be explained, at least in our cases, by the concomitant state of shock. Contrariwise, the acidosis of cases undergoing aggravation is apparently not of the lactic acid type and probably reflects other, as yet poorly understood mechanisms.

Granulomatous hepatitis and cholangiolitis induced by hypersensitivity to procainamide and prajmalium bitartrate

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Two patients with acute myocardial infarction and ventricular arrhythmias developed hepatic injury, one following the administration of procainamide and the other prajmalium bitartrate.

The patient treated by procainamide exhibited fever and chills and the laboratory results demonstrated an increased erythrocyte sedimentation rate, elevated levels of bilirubin, alkaline phosphatase, SGOT and SGPT. A liver biopsy showed well-circumscribed epithelioid cell granulomas, without caseation or giant cells. A second liver biopsy two months later was normal. The other patient receiving prajmalium bitartrate developed jaundice, chills and pruritus and the laboratory tests showed marked eosinophilia and the biochemical features of cholestatic jaundice. The liver biopsy disclosed an inflammatory infiltration in the portal spaces and mild proliferation of marginal bile ductules, consistent with cholangiolitis.

In both patients discontinuation of the respective drug resulted in a rapid and spontaneous improvement in the clinical and biochemical findings. Reexposure to the respective agent caused the clinical and biochemical phenomena to reappear in both instances.

Immunological investigations in the first patient showed the presence of serum mast cell sensitizing antibodies (Ig E), using the indirect mast cell degranulation test, but negative results for macrophage migration inhibition and lymphocyte stimulation with procainamide. In the second patient, deposits of Ig G and IgA were detected at the bile canaliculi by fluorescent staining, and the lymphocytes produced migration inhibition factor (MIF) after *in vitro* incubation with prajmalium bitartrate. Lymphocyte stimulation and mast cell degranulation in the presence of prajmalium bitartrate were not demonstrated.

Granulomatous hepatitis resulting from a chemical is rare. It is generally considered to be a delayed type hypersensitivity reaction. However, *in vitro* tests for cell mediated immunity against procainamide were negative. Since

immune complexes have been demonstrated to induce granulomas in experimental animals (1), it is possible that in our case humoral immunogenic mechanisms were implicated in the disease process.

Little is known about the mechanism of drug-induced cholestasis. Alterations in bile salt metabolism have been suggested (2). We were able to demonstrate immunoglobulin deposits (Ig G and IgA) at the bile caniculi and the production of MIF by the patient's lymphocytes in the presence of prajmalium bitartrate. Thus indirect evidence is provided that immune mechanism were operative in the pathogenesis of the cholestatic jaundice.

Although the exact immunological sequence of events remains to be elucidated, our data support the view that the liver damages induced by procainamide and prajmalium bitartrate were mediated by immunopathogenic mechanisms.

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Artificial liver and kidney by haemoanadialfiltration

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Although chemical pathways of catabolism in mammals are now well known, physical chemistry of the excretion process of molecules which circulate in plasma weakly bound to proteins carriers still today presents areas neither understood nor appropriately considered for its large involvement in physiology and in pathology. Weakly bound middle molecules are defined according to two main features: molecular weight (MW), from 250 to 10,000 dalton and protein binding, measured as K of association, from 40 to 150.

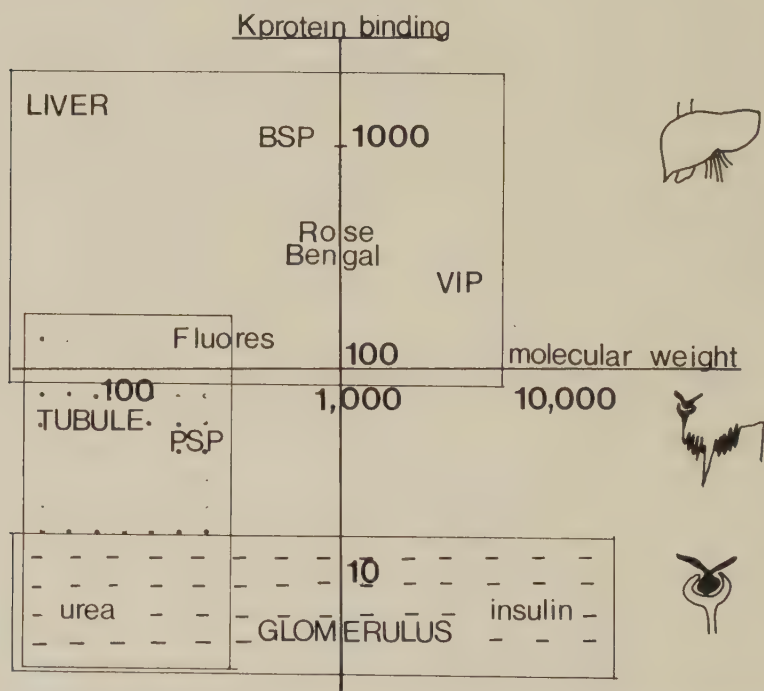


FIG. 1. The world of plasma small and middle molecules according to molecular weight and to protein binding. The excretory way changes especially with increasing K of binding. Molecules which are weakly bound (K 40-150) are excreted both by renal tubule and by liver. These compounds, which comprise hormones, drugs, poisons, toxins, are the most toxic, for their only partial neutralization by serum albumin.

The bidimensional graph of Figure 1 shows the world of molecules classified according these two features. From data already present in the scientific literature defined areas of influence can be attributed to the glomerulus, the tubule and the liver. Tubular and glomerular functions are integrated: glomerular ultrafiltration presents limits of protein binding and the tubule presents limits of molecular weight. Probably also remains an area out of any excretion mechanisms, and which could explain the accumulation of toxic polypeptides during several pathological situations. Haemoanadiafiltration (H-ADF) has been devised for the purpose of clearing from blood weakly bound polypeptides. Blood dilution prior to ultrafiltration step brings to a release of molecules. Experimental basis of H-ADF is shown in the paper "in vitro evaluation of H-ADF for clearance of protein bound hepatic toxins" in this volume.

Clinical work has been conducted with a monitorized equipment shown in Figure 2. Blood is diluted in extracorporeal circulation with balanced salt solu-

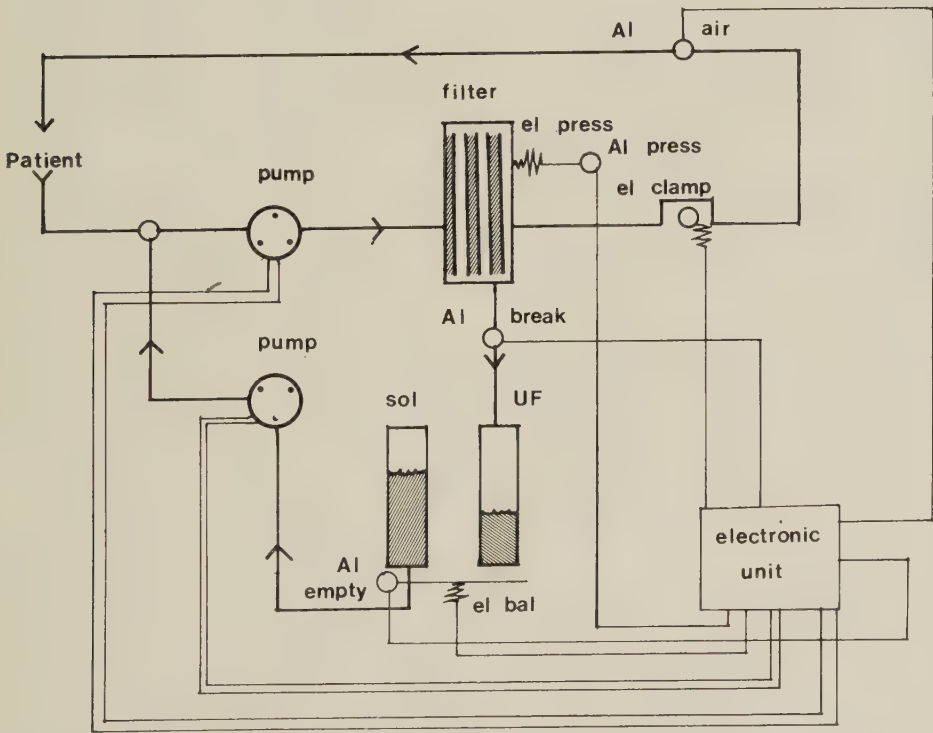


FIG. 2. Electronically monitored haemo-anadiafiltration. Blood circuit shown on the top. Predilution and appropriate ultrafiltration is regulated by a couple: electronic balance, motor-driven clamp. Alarm signals complete the device.

tion and pumped in a high permeability filter (RP6, Rhone Poulenc or TM 30 Amicon). Amount of ultrafiltrate is measured by an electronic balance and regulated, through an electronic unit, by a motor-driven clamp.

CLINICAL EXPERIENCE

We have used haemoanadialfiltration on 15 patients with advanced cancer. The aim of our research was to clear plasma from polypeptides which are bound to globulins probably responsible of the immune depression of this disease. Patients were ambulatory. Low blood flows (50 ml/min) and vein to vein circuit allowed a minimal circulatory risk.

ADF volumes up to ten liters in about 3 hours been tolerated with non disturbances.

CONCLUSIONS

In the future a subtractive pharmacological intervention will cooperate more and more with the classical one, especially in cases of clear middle molecules over charge (Table 1). H-ADF has no biocompatibility problems; it does not induce biochemical alterations. It operates with low blood flow, not requiring an A-V fistula and minimizes cardiocirculatory risks. Its safety is also demonstrated by the clinical use on an ambulatory base.

TABLE 1

Middle molecules in pathology. Hypothesis of a future subtractive pharmacological intervention

Disease	Classical Pharmacological Intervention	Subtractive Intervention
Cancer	Immune stimulation	Removal of blocking factor
Shock	Cortison Antifibrinolytic Adrenalin	Removal of shock polypeptides
Heart failure	Digitalis Vasodilatation Diuresis	Removal of Myocardium depressing factor
Heart failure (post-cardiac surgery)	Cortison Antifibrinolytic Adrenalin	Removal of shock polypeptides
Apudomas		
Insulinomas	Glucose	Removal of Insulin
Gastrinomas		Removal of Gastrin
Argentaffinomas		
Poisoning	General support Antidote	Removal of poison
Liver failure	General support	Removal of toxins

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In-vitro demonstration of cellular immune reactions in patients with drug induced hepatitis and pseudo-LE-syndrome

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8 patients with diphenisatin-induced, CAH-like hepatitis, 2 cases of hepatitis after the intake of analgetics and 2 cases of Venocuran-induced pseudo-LE-syndrome were examined.

Hypersensitivity to the eliciting agents could be demonstrated by measuring the increase in ^3H -thymidine uptake in a 6 day culture of patients own lymphocytes in the presence of sonicated drug.

Using "metabolite containing sera", obtained from normal individuals 90 minutes after oral administration of the effective drug, stimulation was two to three fold higher as compared to the lymphocyte response after addition of the sonicated drug only.

As shown in two follow-up studies there seems to exist only a transient state of drug specific immunity, probably dependent from the type of drug reaction (organ-associated or systemic).

To provide optimal conditions for the *in vitro* demonstration of drug-hypersensitivity, kinetic studies and dose-response curves, therefore, must be established for each drug and each individual.

The lymphocyte transformation test could be of clinical importance in the diagnosis of allergic reactions and in defining the offending agent among a mixture of different substances.

Specificity and characteristic of porphyric hepatopathy

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We call *Porphyric Hepatopathy* (P.H.) the liver disorders observed in *Porphyria Cutanea Tarda* (P.C.T.), in *Porphyria Variegata* (P.V.) and in *Erythropoietic Protoporphyrria* (E.P.). The fundamentals for the Specificity of P.H. are histochemical, pathological, immunological, biochemical and clinical arguments.

1. *Histochemical arguments*: The porphyric hepatocyte has less cytochromoxidase and more TPN than the hepatocyte of alcoholic cirrhosis (1).

2. *Pathological arguments*: In P.H. the *long initial or early stage* of hepatic porphyrinosis, observable by means of fluorescence techniques using Wood's light which may be asymptomatic and not observed in other hepatopathies. It is accompanied by siderosis (absent in E.P.), steatosis (if there is associated ethylism) and initial fibrosis. The Pathology in 41 cases of P.C.T. revealed premature lesion in 14, only porphyrinosis in 5 and porphyrinosis along with siderosis, steatosis and fibrosis in another 9. The *second stage*, that we call *chronic porphyric hepatopathy* (15 cases in our series) with proliferation of Kuppfer's cells and activation of the mesenchyma and infiltration of mononucleated cells in the portae spaces. This stage includes a form of *persistent hepatitis* (9 cases) with portal infiltrates and a form similar to *chronic aggressive hepatitis* (6 cases) with piecemeal necrosis, bridge necrosis and penetration of mononucleated cells in the small lobules. The *third stage* is micronodular (3 cases) or macromicronodular (9 cases) *porphyric cirrhosis*. Out of the 12 porphyric cirrhosis, we observed *hepatoma* in 5 and *hemochromatosis* in 3.

In E.P. (4 cases) none was alcoholic and we observed porphyrinosis in 2, fibrosis and portal infiltrates in 1 and macromicronodular cirrhosis in 1. In one, non alcoholic case of P.V., we observed hepatic porphyrinosis. We have no experience with electronic microscopy. There appear to be premature mitochondrial, microcrystalline inclusions (2).

3. *Biochemical and clinical arguments*:

a) Decreased uroporphyrinogen decarboxylase activity in the liver in P.C.T. (3) and experimental porphyria in the rat with hexachlorobenzene (HCB) (4).

b) Peculiar porphyrin deposit (Uroporphyrinal and Heptacarboxylporphyrin III) in the liver in P.C.T. (5) and *typical urinary elimination* of porphyrin of 7, 6 and 5 carboxiles in P.V. (6); uroporphyrin always greater than the coproporphyrin (7) and of heptacarboxylporphyrin III in P.C.T. (8). *Faecal elimination* of Elder's isocoproporphyrins in P.C.T. and in experimental porphyria in the rat with HCB (9).

c) *Not all the porphyrics are ethylic*. In 4 cases of E.P. and in 4 of P.C.T. there was no ethylism and they develop P.H. Alcohol exteriorizes the hereditary disturb (10) (7). Not all chronic alcoholics become porphyric. Only 0.8% of hepatic cirrhosis finish in P.C.T. (11).

d) *Evolutionary differences* between P.H. and alcoholic and cirrhotic hepatopathies: Initial, asymptomatic stage with hepatic porphyrinosis. Longer development with less complications. Higher incidence of *hemochromatosis* and frequent association with *erythrocytosis*. Few functional differences: Only increased BSP retention (12) (13).

4. *Immunological arguments*: We have observed (14) (Table 1) by comparing the results in 30 patients with P.C.T. with those in 20 non-alcoholic porphyric patients: a) Significant increase in IgA ($p < 0.05$) and in IgM ($p < 0.05$) in the porphyric cases. b) Antimitochondrial antibodies in 40% of the porphyric cases and in none of the non-porphyrin alcoholics. c) Increase in the B Lymphocytes in porphyrics ($p < 0.025$). d) That the P.C.T. is significantly associated with the antigens HL-A 11 and HL-A 28 in the first series and HL-A 14 in the second series and less frequently HL-A 10 ($p < 0.025$) in the first series and HL-A 27 ($p < 0.01$) and W 10 ($p < 0.01$) than in the general population in Spain. The risk of suffering from P.C.T. is more than three times greater in HL-A 3 and HL-A 28 groups.

TABLE 1

Immunological arguments

<i>No. cases</i>	<i>P.C.T.</i>		<i>t</i>	<i>p</i>	<i>Etilics without Porphyria</i>	
		<i>30</i>				<i>20</i>
IgA	x	430.9 mg%	1.82	< 0.05	x	325 mg%
IgM	x	200.7 mg%	1.74	< 0.05	x	169.5 mg%
IgG	x	1584.6 mg%	0.65	NS	x	166.7 mg%
Antib. mitoch.		+12 cases	—	< 0.0005		all negatives
Lymphocytes B	no.	566.2	2.72	< 0.005	no.	435.7
Rosettes B	%	32.9	2.29	< 0.025	%	27.1
Lymphocytes T	no.	1021.8	0.32	NS	no.	998.4
Rosettes T	%	59.03	—1.18	NS	%	62.4

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Transient mixed cryoglobulinemia in typhoid hepatitis and in CML

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Cryoglobulins are abnormal serum proteins or protein complexes, which undergo reversible precipitation at low temperatures. Despite the obvious clinical manifestations, including hyperviscosity and precipitation in blood vessels, the cause of the precipitation in cold temperatures is unknown, and might be attributed to some basic structural disorders of the proteins (Middaugh 1977). Lerner *et al.*, (1947) found cryoglobulins to be immunoglobulins, and Meltzer *et al.*, (1966) have classified cryoglobulins according to their being monoclonal (IgG or IgM) or mixed (IgG-IgM). In certain instances the cryoglobulins may disappear spontaneously following treatment (transient cryoglobulins) (Biró 1967). We presently describe two patients with transient mixed cryoglobulinemia, one associated with severe typhoid hepatitis and the other with chronic myelocytic leukemia.

In the first patient, a 28 year old clerk, the cryoglobulin appeared simultaneously with salmonella typhi bacteremia, severe hepatic damage and a positive Widal test, and disappeared when the patient recovered. The well known association between liver diseases and cryoglobulinemia (Levo 1977), supports our hypothesis that the hepatic reticuloendothelial system might be responsible for a special "processing" of the Salmonella bacilli, thus inducing the synthesis of an antibody with the physicochemical characteristics of a cryoglobulin.

In the second patient, an eighty year old man the chronic myeloid leukemia (CML) was associated with mixed cryoglobulinemia, and when the patient entered into remission following treatment with busulfan the cryoglobulin disappeared. Mixed cryoglobulinemia had been successfully treated by cytotoxic drugs (Biró 1965), but the disappearance of a CML associated cryoglobulinemia to the best of our knowledge has not been hitherto reported.

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Medroxyprogesterone and autoimmune liver disease

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Effect of medroxyprogesterone acetate on the clinical course of 15 patients with primary biliary cirrhosis and chronic active hepatitis was investigated. The response in all subjects was good: the subjective symptoms decreased, the liver function tests showed a tendency towards normal values. Furthermore, the levels of serum antibodies declined and the liver metabolic ability, as tested by serum albumin levels and indices of drug metabolism, was impaired. The beneficial influence of the progesterone on the patients might be due to the immunosuppressive effect and enhancement of protein synthesis, but to clarify the mechanism further studies are indicated. Our results suggest that medroxyprogesterone might be a valuable alternative for patients with autoimmune liver disease, particularly in cases developing resistance or unwanted reactions to previous therapy.

Hepatocellular carcinoma and hepatitis B virus

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Hepatocellular carcinoma (HCC), postnecrotic cirrhosis, chronic hepatitis and viral hepatitis are prevalent in Taiwan. The age-adjusted death rate of HCC per 100,000 population in male in 1975 was 25.2%. High prevalence rates of HBs antigenemia in healthy adults has been demonstrated in Taiwan up to 17.8% by radioimmunoassay. To clarify the relationship between hepatitis B virus (HBV) and HCC, we have done a series of studies. This communication is to present some of the results of our studies.

The prevalence rates of HBsAg in sera studied by reversed passive hemagglutination (RPHA) were 81.9% in 149 cases of HCC, 81.6% in 76 cases of cirrhosis, 89.8% in 98 cases of chronic active hepatitis and 41.3% in 46 cases of acute viral hepatitis. In contrast, the rates were 12.5% in 81 cases of other malignancies, 14.3% in 7 cases of alcoholic hepatitis, none in 2 cases of lupoid hepatitis, 20% in 5 cases of drug-induced hepatitis, 8.5% in 47 cases of miscellaneous liver disease and 11.2% in 1,966 healthy adults. From these results it is clear that HBV infection is the cause of cirrhosis and chronic hepatitis in most cases in Taiwan, and it is likely that this kind of infection might be the cause or one of the main causes of HCC.

When the concentration of HBsAg in sera was studied by RPHA, it was significantly lower in patients of HCC and cirrhosis. On the other hand, in the healthy adults, it was significantly lower in those above 39 years of age. It has been noticed repeatedly that once HBsAg is detected during serological survey in the normal people, the antigenemia tends to be persistent. However, it might be possible that the gradual decrease of HBsAg prevalence in elder persons is partly due to elimination of the chronically infected HBV and partly due to the decrease of serum HBsAg concentration to a level not able to detect by the presently available methods. If it were true, the low concentration of HBsAg in serum of patients with HCC and cirrhosis might be due to that the infection of HBV had developed in very early life in these patients.

The concentration of anti-HBs was studied. It was lower in HCC and cirrhosis than that in other malignancies and healthy adults. In healthy adults, it was

significantly lower in those of age above 39. These phenomenon might be due to the change of immunological response to long stimulation.

HBV infection in parents of HCC patients with HBs antigenemia was studied. Eight or 73% out of 11 mothers had HBsAg in serum with concordance in subtype with their children, and the remainder 3 HBsAg-negative mothers all had anti-HBs. These results show the important role of mother in the HBV transmission in Taiwan where adw is the predominant subtype, and indicate that the transmission of HBV to children from mother develops in early life.

HBsAg in liver cells was studied by orcein stain in 86 cases of liver disease. Among 19 cases of HCC coexisted with cirrhosis, HBsAg was demonstrated in cytoplasm of liver cells of cirrhotic part in 9 cases and in serum in 17 cases. In 6 cases of HCC without cirrhosis, HBsAg in liver tissue was studied by immunofluorescence. HBsAg was demonstrated in cytoplasm of liver cells of non-neoplastic part in 4 cases with serum HBsAg RPHA titer above 16. The specimens in this study were obtained from the surgically resected specimen for curable treatment. The patients were still well and the non-neoplastic parts of liver were histologically normal. Therefore, these patient might be asymptomatic carrier of HBsAg for a long time before the development of HCC.

As a conclusion, there are many evidences suggesting that a male together with HBs antigenemia and chronic hepatitis or cirrhosis has higher risk to develop HCC.

Data about cholelithiasis: A diagnostic triad

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Acute abdominal catastrophes are often due to cholelithiasis. The patients are mostly those who have not been operated upon at the early stage.

The doctor's dilemma in referring the patient to surgery is accentuated when the gallbladder's peroral filling was absent and repeated examination seemed to be necessary to strengthen diagnosis. Costly weeks and months elapsed till repeated intravenous cholegraphies or typical gallstone colica might suggest the same diagnosis and surgery became obligatory.

Authors collected a ten-year series of gallstone diseases in the Zirc County Hospital, during this period 353 cases were subjected to cholecystography altogether. Peroral study was effective, with good filling in 128 cases, non-filling in nearly the double number: 213 patients. The filled 128 gallbladders contained negative stone shadows in 99, positive ones in 29 cases.

The patients with non-filling of the gallbladder underwent duodenal tubing connected with study of the biliary secretion. Duodenal tubing was repeatedly done and always connected with lavage through duodenal application of 30% magnesium sulfuric solution, or posterior lobe hypophys. extract injection. The B fraction of the bile was unanimously absent in all 213 non-filling cases, even upon repeated studies.

Upon convincing persuasive consent 93% of the gallstone patients underwent surgery, 315 patients altogether. Surgery revealed biliary stones in all but one patient, the latter had a tortuous cystic duct and chronic cholecystitis.

Distribution of cholelithiasis in ten years:

Diagnosed altogether	353	cases
Peroral nonfilling	213	»
Filling with stone shadows	128	»
Early surgical intervention	315	»
Gallstone verified	314	»

Authors' series of the above cases was suggestive enough to state a triad which is characteristic in gallbladder stones as follows:

1. Gallbladder non-filling;
2. Intact hepatocellular function;
3. Absence of B bladder-bile upon repeated duodenal tubing.

Due to the relevant practice, the number of surgically treated gallstone patients became afterwards. The surgery revealed then 50-60 gallstone diseases yearly, instead of the earlier 20-30 cases per year. Most important is that even those patients were referred to surgery whose gallbladder showed non-filling upon the first attempt. Repeated studies became but exceptions, hospital care was shortened, diagnostic costs cut lower.

The treble symptoms called as triad is diagnostic for gallbladder stone disease, and authors' practice goes on still strenghtening the statements as shown above. The repeated studies are not only costly and time-consuming, but also obsolete and unsuccessful, according to the present authors' findings. The often not well tolerated intravenous cholegraphy can also be avoided in most cases.

Other arguments in favour of the strategy suggested here is that mortality of the early and "à froid" cholecystectomies is considerably less than the danger of empyema or pancreatitis in cases not operated in due time. Operative mortality of early cholecystectomy is less than 1%, that of late surgery in presence of complications may be as high as 6-27%. It is reasonable to suggest early surgery therefore. There was not a single operative loss in the authors' series of 315.

It can be stated therefore that early surgery helps to avoid or lessen high mortality, cholecystitis, empyema vesicae felleae, biliary peritonitis, perforation, pancreatitis, cholangiohepatitis and other secondary diseases. The disclosed diagnostic signs, collected and called gallstonic triad is rendered to alleviate diagnosis and early and relevant therapy.

SUMMARY

Authors establish after ten years of observation that the diagnostic triad indicates cholelithiasis if the clinical symptoms give also evidence of cholelithiasis.

1. If the gall-bladder does not fill with contrast substance.

2. The liver function tests are negative.

3. The B bile does not evacuate when probing repeatedly. The complaint may be only a slight feeling of epigastric pressure or also tympanites. It follows from the above-mentioned symptoms that in these cases it is unnecessary the repetition of the cholecystographia neither per os nor intravenously or by infusion. This process is of diagnostic and economic importance.

Early operation is important, because it results in a rare occurrence of complications afterwards. In this way the hospital treatment will be shorter of duration. Out of the 315 cases there were not any exits including even aged patients. According to the other statistics as well exit is under one per cent. The risk of an early operation is reduced as opposed to the peril of a later performed one.

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Decrease hepatic and RBC uroporphyrinogen-decarboxylase activity (URO-DEC) as marker of porphyria cutanea tarda (PCT)

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Porphyria cutanea tarda (PCT) has long been considered as an acquired disorder of porphyrin metabolism. Most cases of PCT occur sporadically and the clinical and biochemical syndrome is seen as a complication of liver-cell damage, more frequently induced by iron excess and/or alcohol overconsumption.

However a familial occurrence of PCT and abnormalities of porphyrin metabolism in apparently unaffected relatives of patients have been reported. These findings suggest a genetically determined contribution to the pathogenesis of PCT, likely as an enzyme defect with different penetrance, which could induce an inherited predisposition to react to iron, alcohol and other factors.

Kushner *et al.* (1,2) first showed a decrease in hepatic URO-DEC activity assayed by incubating tritiated PBG with liver homogenate mostly from surgical biopsies. Moreover, they found a significative decrease in URO-DEC activity also in erythrocytes of patients with PCT and their relatives. This finding suggested that PCT may be inherited as an autosomal dominant trait.

More recently, Elder *et al.* (3), using the pentacarboxylic porphyrinogen as substrate confirmed the decrease in hepatic URO-DEC activity, but failed to confirm the same enzyme defect in RBC of patients with PCT.

The aim of our study was to investigate URO-DEC activity in Liver and RBC of patients with PCT.

Hemolysate and liver homogenate (supernates) from needle biopsy taken during laparoscopy (5-15mg) were incubated at 37°C with unlabeled PBG (in TRIS-HCl 0.1M buffer, pH 6.8) as substrate.

Incubation lasted 30 min. for RBC, accordingly to Grandchamp (4) and 6 hours for liver supernate, with minor modifications of the method of Kushner (1). 8-7-6-5-4-carboxylic porphyrinogens after incubation and methylation accordingly to Doss (5), were detected by TLC and direct spectrofluorimetric scanning of the plate (Zeiss PMQ II Spectrophotometer with Chromatoscanner).

URO-DEC activity was inferred from the amount of the different carboxylic compounds expressed as percent of the total synthesized porphyrins. 16 patients with PCT, confirmed on the clinical and urinary porphyrin pattern, and 16 male controls were investigated.

Normal human liver was obtained at laparotomy from patients undergoing surgery for a variety of benign digestive disorders.

The results are summarized in the Table

	Subjects	8-COOH %	7-COOH %	6-COOH %	5-COOH %	4-COOH %
LIVER	Contr. (6)	67.5 ± 11	7.4 ± 2	1.7 ± 1	5.4 ± 3	17.8 ± 10
	PCT (8)	78.0 ± 10	18.4 ± 10	0.9 ± 0.8	0.7 ± 0.5	2.4 ± 2.5
	Anova (P)	N.S.	< 0.05	N.S.	< 0.001	< 0.01
RBC	Contr. (16)	4.8 ± 1.4	7.0 ± 1.6	2.2 ± 0.6	3.7 ± 1.3	82.3 ± 3.9
	PCT (16)	7.4 ± 1.5	10.5 ± 1.9	3.6 ± 1.0	4.0 ± 1.2	74.2 ± 3.5
	Anova (P)	< 0.001	< 0.001	< 0.001	N.S.	< 0.001

CONCLUSION

Our simplified method seems to be sensitive as the previous ones (radioenzymatic or not) in detecting the defect of URO-DEC activity in both liver and RBC of patients with PCT. The detection of enzyme defect even in RBC may be considered a further evidence that the decrease in URO-DEC activity is a genetic marker of PCT.

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Diagnostic value of the intravenous galactose tolerance test in different alcoholic hepatopathies

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THEME OF STUDY

The intravenous galactose tolerance test was carried out in 16 patients with Acute Alcoholic Hepatitis and in 37 patients with Alcoholic Cirrhosis of the liver, who all had histological confirmation (through a liver puncture biopsy), to establish the possible comparative value of the test as opposed to the liver puncture biopsy and the other hepatocellular function test.

METHODS

The method of enzymatic determination of galactose of Tengstrom was followed.

The following parameters of liver function were also studied:

Prothrombin time, Albumine, B.S.P. in anicteric patients.

RESULTS

1. The half time of galactose in normal patients was 15 ± 3.03
2. The half time of galactose in patients with A.A.H. was 16.53 ± 1.89
3. The average half time in patients with cirrhosis was 50.13 ± 30.2

There were a highly significant T $1/2$ correlation between the patients with A.A.H. and A.H.C. (p 0.01)

There were no statistically significant correlation when the other parameters were compared.

CONCLUSIONS

1. The half time of galactose in A.A.H. is hardly affected.
2. The half time is always affected in alcoholic cirrhosis of the liver and is related to the degree of hepatic injury.
3. The I.G.T.T. is the only liver function test useful in the differential diagnosis of A.A.H. and A.H.C.

FREE PAPERS
GASTROENTEROLOGY

Primary cholerhoeic enteropathy

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Recently a new bile acid diarrhogenic syndrome, without evidence of ileal involvement was described (1): a genetic or acquired defect in the ileal bile acid carrier mediated transport system or a primitively increased hepatic bile acid synthesis, saturating the transport system, were considered the two possible mechanisms responsible for the bile acid malabsorption (BAM).

The present investigation was carried out on 50 subjects with chronic diarrhoea (group 1), on patients with definite BAM (11 with ileal resection, group 3, 7 with jejunum-ileal by-pass, group 2) and on 10 control subjects (group 4).

The 50 patients with diarrhoea had no radiological, biochemical, histological evidence of ileal involvement, no signs of neuro-hormonal disease, which might have caused the diarrhoea.

BAM was evaluated by means of cholestyramine $1\text{-}^{14}\text{C}$ glycine breath test + ^{14}C recovery in stools (3), and by bile acid daily faecal output (by gas-liquid-chromatography). In 5 controls and 5 diarrhoea patients in whom BAM was confirmed, the concentration of bile acids in the aqueous phase of stools (after ultracentrifugation at 100,000 g) was evaluated. Results (mean \pm SD):

	<i>Faecal weight g/day</i>	<i>Breath $^{14}\text{CO}_2$ output in 6 h % dose</i>	<i>Faecal ^{14}C corrected for ^{51}Cr</i>	<i>Faecal Total B.A. (mg/day)</i>
Group 1	250 \pm 200 p < 0.01	3.4 \pm 2.7 p = ns	17 \pm 20 p = ns	348 \pm 300 p = ns
Group 2	668 \pm 292 p < 0.001	8.9 \pm 7.2 p < 0.05	32 \pm 18 p < 0.005	1232 \pm 400 p < 0.001
Group 3	498 \pm 278 p < 0.001	15.0 \pm 5.0 p < 0.001	28 \pm 12 p < 0.001	889 \pm 347 p < 0.001
Group 4	90 \pm 50	2.6 \pm 0.6	3 \pm 2	226 \pm 70

In group 1 only 5 patients had both abnormal $^{14}\text{CO}_2$ in breath and ^{14}C in stools. When elevated ^{14}C in stools was observed in presence of a normal breath, it was ascribed to increased transit time.

Qualitative analysis of faecal bile acids revealed the presence of primary bile acids in group 1, 2, 3, but only traces in group 4. The 5 patients with BAM (increased $^{14}\text{CO}_2$ in breath, increased faecal ^{14}C , increased faecal bile acid output) presented in the aqueous phase of the stools a concentration of dihydroxy $> 3 \text{ mM}$ (in 5 controls only negligible amounts were found).

Conclusions: only a minor percentage of the patients with chronic diarrhoea show BAM. These may be considered primitive bile acid malabsorption patients, having no signs of ileal involvement. The presence of primary bile acids in stools, thought pathological may be observed also when increased transit time occurs.

In patients with primary BAM the role of bile acid in determining the diarrhoea is confirmed by a $> 3 \text{ mM}$ concentration (2) of dihydroxy in the aqueous phase of stools.

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Role of hypersensitivity-related phenomena in enteric pathology

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It has been excluded that gluten may have any relevant toxic action on the enteric mucosa in the pathogenesis of the glutensensitive-enteropathy (GSE) (1). Nevertheless, there is compelling evidence that immune mechanisms play a role in GSE (1). The presence of circulating anti-gluten antibodies (2), the behaviour of plasma complement and IgA levels (3), the B-cell differentiation and deposition of immune complexes (IC) in the mucosa, the appearance of lymphocytes in intraepithelial sites in previously inactive GSE after gluten challenge (4, 5), may be all suggestive for an immunologic involvement. Several reports (6, 7) have dealt with immunological studies of the colon in ulcerative colitis (U.C.) and Crohn's disease (C.D.), suggesting immune alterations in these diseases. Immediate hypersensitivity-related phenomena to dietary antigens were postulated many years ago in GSE, U.C. and C.D. on the basis of clinical improvement following withdrawal of alleged antigens and recurrence following their reintroduction. Conclusive evidence was never obtained because the techniques available such as skin testing were considered unreliable (6). In order to study immediate hypersensitivity, we performed basophil counts in 7 patients with GSE, 5 with U.C. and 5 with C.D., by using a simple, one-step technique, which metachromatically stains basophils in whole blood (8, 9). *In-vitro* basophil degranulation tests (8), performed with increasing concentrations (0.1 to 400 ug/ml) of the alleged antigens (gluten and milk), were carried out by counting basophils before and after exposure to antigens. Concomitant Platelet-Activating-Factor (PAF) and histamine release were assayed (9). In 7 patients basophil counts was 7 ± 6 (N.V.: 40 ± 15) and PAF blood reservoirs were low

	<i>Controls</i>	<i>GSE</i>	<i>U.C.</i>	<i>C.D.</i>
No. cases	10 %	7 %	5 %	5 %
Gluten	20 ± 10	61 ± 16	83 ± 19	50 ± 20
Milk	22 ± 8	40 ± 10	63 ± 9	53 ± 9

during the acute phases of the diseases, as expression of an *in-vivo* degranulation. *In vitro*, IgE sensitization of basophils to gluten and milk was detectable in 20 patients concomitant with PAF release.

The table summarizes the results concerning gluten—and milk—induced basophil degranulation (mean \pm SD). The tests were considered positive when the percentage of degranulation was over 30%. Patients with low basophil counts showed a marked increase when on an antigen—free diet. Surgically—resected enteric specimens from patients with U.C. and C.D. contained numerous degranulating mastocytes in the diseased submucosa and muscle layers. Mediators released from degranulating basophils and mastocytes upon challenge with specific antigen, are implicated in tissue injury. Among them, PAF has been (10) shown to involve platelets as the natural amplifying mechanism of the IgE—basophil system. The end—result is enhanced vascular permeability and inflammation. These data suggest the involvement in enteric pathology of the IgE-basophil-PAF system. Furthermore, it has recently been suggested that basophil and mastocyte degranulation may trigger IC vascular deposition (10, 11).

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Acute pancreatitis: prognosis and therapy

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Complicated acute pancreatitis has a poor prognosis. In a retrograde study 55 patients with acute pancreatitis were reviewed in order to establish the prognostic value of eleven indices which were determined either at admission (age, white blood cell count, blood glucose, SGOT, LDH) or 48 hours after the onset of the disease (hematocrit, serum calcium, base excess, BUN, fluid repention, pO_2). The results show: 1. the five indices determined at admission do not permit a clear identification of the variable courses of acute pancreatitis, 2. but a high risk group can be selected prior the manifestation of complications such as shock, pulmonary and renal insufficiency; in this group an early intensive care is recommended within the first 24 hours after the onset of the disease i.e. a vigorous fluid replacement, endotracheal intubation and assisted ventilation with PEEP, and if necessary peritoneal dialysis.

Furthermore these indices are helpful to decide very early whether a patient has to be transmitted to a medical centre for intensive care and/or surgical treatment, 3.48 hours after the onset of acute pancreatitis intensive care produces more or less no effect.

Maintenance cimetidine treatment in duodenal ulcer

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A four weeks treatment with cimetidine 1 g/day obtained endoscopic healing in 104 patients with duodenal ulcer; all healed patients were symptom-free.

They all were randomly given three different maintenance therapies: cimetidine 400/mg nocte for nine months (64 cases) or for twelve months (20 cases) and Maalox 0.6 gx4/day for twelve months (20 cases).

Relapse rate at twelve months after healing was determined with endoscopy.

No significant difference between cimetidine groups was detected: 24% versus 25%. A higher relapse rate with a marked significance in Maalox group was observed (48%).

Malabsorption associated with "acquired hypogammaglobulinemias"

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INTRODUCTION

Congenital agammaglobulinemia was first described by Bruton (1) in 1952. Not long after, it became apparent that various syndromes of acquired hypogammaglobulinemia are much more frequent and that gastrointestinal disturbances are common findings in the immunoglobulin deficiency syndromes (2, 3).

A few years later numerous reports of acquired hypogammaglobulinemia and its association with nodular lymphoid hyperplasia, diarrhea, achlorhidria, splenomegaly, tonsillar hypertrophy, eczematoid dermatitis, carcinoma of the stomach or colon, thymomas and acute pancreatitis were described. Infestation with giardia lamblia and IgA deficiency is a common observation in these patients (4, 5, 6).

Dysgammaglobulinemia would be documented more often if serum immunoglobulins were quantified routinely in patients with diarrhea and malabsorption, routine serum protein electrophoresis may be an adequate screening procedure for the detection of some of these immunoglobulins aberrations.

Case presentation

Male forty seven years, first seen at the University Clinic of Navarra for evaluation of chronic diarrhea. He presented a history of recurrent upper respiratory tract infections, bacterial pneumonias and typhoid fever. Since 1971 chronic diarrhea was established, which in the last 9 months was more severe (10-12 depositions/day). Other symptoms were: malaise, lassitude, crampy pain in lower extremities and shoulders, and a weight loss of 14 kg.

Analitical data: red and white count, hemoglobin, prothrombin index and calcium were normal. It was detected a polyclonal hypogammaglobulinemia of 0.65 gr% with an IgG of 490 mgr%, IgA 46 mg%, IgM 42 mgr%. The total isolated lymphocytes were 641 mm³, T lymphocytes 70%, B lymphocytes 36% and T active lymphocytes 8%. The indirect immunofluorescence was normal. The dextrose test was 20% and 24 mg/dl in urine and plasma respectively.

Schilling test was altered less than 8% and the B-A-O was O with a M.A.O. of 9'22 mEq/60 min. *Giardia Lamblia* was isolated in the stools.

Gastrointestinal x-ray films, gastric, small bowel and rectum biopsies were performed. The small bowel transit showed: 1. Malabsorption pattern. 2. Multiple nodular defects and 3. Inflammatory changes. Atrophic gastritis was seen at endoscopy with a gastric biopsy which showed enteric metaplasia. The peroral small bowel biopsy showed normal villous architecture with a greater than normal height compared to that observed with normal caliciform cells, lack of inflammatory cells and a striking decrease of plasmatic cells in methyl pyronine green stain in small intestine, gastric and rectal biopsies; biopsy, also evidence nodular lymphoid hyperplasia, with a normal mucosa appearance at the sigmoidoscopy.

DISCUSSION

Hypogammaglobulinemia in adults is almost always of the "acquired" variety. The cause for immunoglobulins decrease is still unknown. Agammaglobulinemia in children presents with poorly developed tonsillar lymphoid tissue and absence of Peyer's patches, while acquired hypogammaglobulinemia frequently presents hypertrophic tonsillar tissue and the nodular hyperplasia. This could possibly be due to the existence of suppressor T cells, which inhibit B cell transformation to plasma cells, an intrinsic defect in B cells which results in a failure of these to mature (7, 6).

Our patient had a low lymphocytic count and a global decrease in immunoglobulins with a lack of plasma cells in intestinal biopsies as we have mentioned above.

Hypogammaglobulinemic patients with and without NLH, do not have distinctive characteristics. NLH appears to be a particular reaction of the Gastrointestinal tract in acquired hypogammaglobulinemia and involves B cells impairment with normally functioning T cells. We did not observe NLH in our histological study of the small intestine, although radiologically there seemed to be some degree of NLH. The absence of histological evidence of NLH in our study can possibly be due to the patchy distribution of such nodules like most authors suggested (8, 9, 10, 11, 12).

In the congenital form diarrhea and malabsorption are infrequent and arthritis is a common finding. Cause of diarrhea is unknown and some authors suggest overgrowth of the normal bacterial flora, salmonellas, shigellas or giardias; why this alteration is not observed in the congenital form where the humoral defense system is greatly altered is yet to be explained.

Achlorydria and secondary bacterial overgrowth are another factor which could explain the presence of diarrhea. Pancreatitis may also have pathogenetic

role. Colitis was associated in these patients with malabsorption indicating that both, small and large bowels, were involved. In others patients no malabsorption could be demonstrated and small bowel biopsies showed normal villus architecture. According to the clasification of the Symposium on Pathology of Gastrointestinal tract, our case could not be ubicated in any of the five groups, because this patient has a normal villus architecture with a diminution of the plasma cells within the lamina propria and a polyclonal hypogammaglobulinemia (13).

Treatment with human gammaglobuline at a dose of 125 mg/daily for 4 days was performed (6). Treatment was continuous administrating 125 mg of human gammaglobulin at 3 weeks intervals. The patient's evolution was excellent: he is presently doing well.

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Cimetidine in the treatment of peptic ulcer.

A double blind study

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Fifty-eight patients with duodenal or pyloric canal ulcer and 31 patients with gastric ulcer, all endoscopically confirmed, entered a double blind outpatient trial. Three groups were established on the basis of the severity of the ulcer disease: mild, moderate and severe. Age, duration of the disease, intensity of the ulcer symptoms, previous complications (haemorrhage, perforation, pyloric stenosis) and associated diseases were estimated by means of a score system. The patients received either 1 g of cimetidine per day or placebo for 6 weeks. Both groups of patients received antacids for symptom relief only. Endoscopy was repeated at week 6 and healing was defined as being free from craters and erosion.

In the duodenal ulcer, at the end of 6 weeks of therapy, 60% of the cimetidine-treated patients, and 45% of the patients treated with placebo showed evidence of ulcer healing ($p > 0.20$). Both groups consumed the same amount of antacids before beginning the study. During the 6 weeks treatment the cimetidine group consumed a smaller amount of alkalines than the placebo group ($p < 0.005$). After a year of finalising treatment there is no significant difference between the two groups with regards to healing persistence.

These results differ from those obtained in other studies (2, 3, 4) (Fig. 1). The reason for this could lie in the difference in consumption of antacids by the patients who have healed (4, 5). Our placebo group consumed the same amount of alkalines as the American placebo group, which was clearly higher than that consumed by the European placebo group and that administered to those patients treated with cimetidine in any of the trials.

34% of our patients have "severe ulcer disease", 52% have "moderate ulcer disease" and only 14% have "mild ulcer disease". Can these patients be compared, with regards to severity of the disease, to those of other trials? We believe that this possible variation might explain why our healing percentage is lower. Nevertheless, it would be useful to assess the severity of the ulcer disease with uniform criteria.

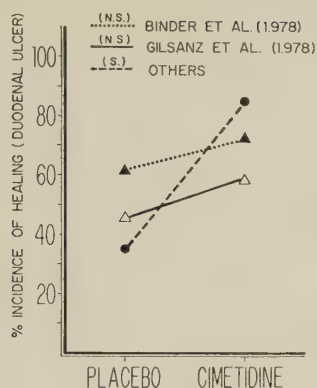


FIG. 1. Healing incidence in Groups of Patients with Duodenal Ulcer after six weeks of placebo or cimetidine therapy.

In the simple gastric ulcer, cimetidine has proved to be more efficacious than placebo after 6 weeks of treatment, ($p < 0.01$). However, this is not so in the case of multiple gastric ulcers, where there is no significant difference between the results.

Healing persists to practically the same degree in the patients healed in both groups up to one year after finalising treatment. No untoward effects were observed during cimetidine treatment.

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A new pathogenetic hypothesis on the duodenal peptic lesion

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The endoscopy demonstrated how the duodenal lesion often appears and heals at the same time with the clinical symptoms. In a previous work (1) we have observed an annual secretory biorhythm. On February-March and October-November a lower pH and a higher rise in basal acid output (B.A.O.) were found in ulcerous duodenal patients and in hypersecretory individuals without ulcer than in normal subjects. Another study (2) showed that the hypersecretion and the lowered pH produced a functional reduction in the pyloric diameter with an increase in the time it remains closed. Such reduction produced a greater number of antral contractions necessary for gastric emptying. With the use of a computer, we were able to demonstrate a decrease in gastric juice output for each antral contraction in the hypersecretory patients. A 96.5% reduction was found in respect to the normal output. Therefore a squirt-like gastric flow enters an almost empty bulb striking the bulbar wall facing the pyloric axis. The persistence of this occurrence very likely produces a peptic lesion. It could be worsened by the presence of the activated pepsin by lowered pH. We think that the lesion is due to annual variations in basal secretory output to the lowered pH and to the reduction of pyloric diameter. The periodicity, the only localization, the usual bulbar localization and the reappearance of the peptic lesion on the same wall support this hypothesis, which could clarify some unresolved questions about pathogenesis of duodenal ulcer disease.

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Endotoxic shock after surgical treatment of small intestine volvulus, a new syndrome

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Following the experimental work of Fine *et al.* (1-2), aimed at creating an endotoxic shock secondary to intestinal ischemia, we were able to demonstrate clinically a perfectly analogous situation of human endotoxaemia after intestinal volvulus with strangulation and rather severe ischemia.

Four subjects were studied (Table 1). Shortly after operation within the time intervals indicated in Table 2, they each presented an intense endotoxic shock syndrome with negative blood and peritoneal fluid cultures, implying a characteristic polyvisceral failure (Table 3).

TABLE 1

age	sex	stay	evolution	antecedents
44	♂	2 days	dead	gastrectomy
53	♀	2 d.	dead	appendicectomy
44	♂	24 d.	alive	gastrectomy
57	♂	29 d.	alive	gastrectomy by pass

TABLE 2

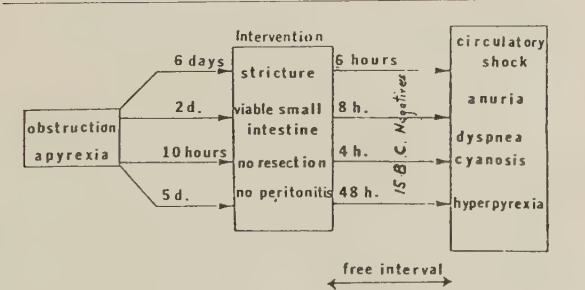
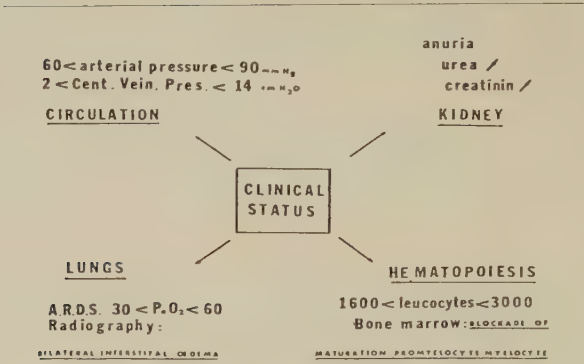


TABLE 3



The physiopathology of this syndrome, having been amply demonstrated (Fig. 1), allows us to envisage a logical course of treatment (3):

Elimination of circulating endotoxins (Exchange Transfusion or Plasma-pheresis).

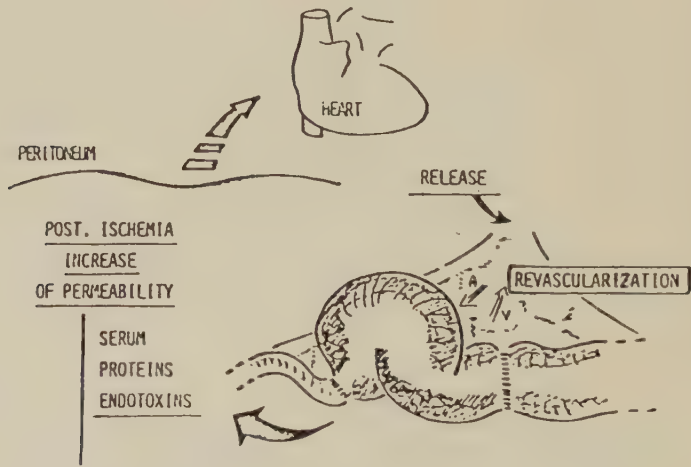


FIG. 1

Elimination of free intraperitoneal endotoxins (Peritoneal lavage).

The first 2 patients died in spite of energetic symptomatic treatment (Table 4).

The other 2 patients survived (Table 5) thanks to an adapted therapy according to the principles described above. The presence of circulating endotoxins

TABLE 4

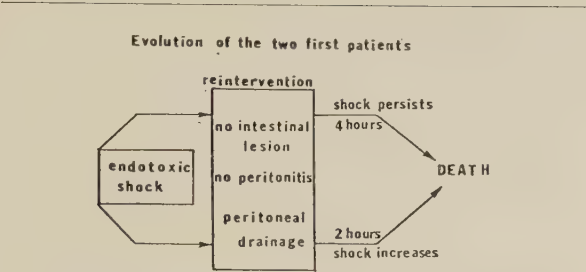
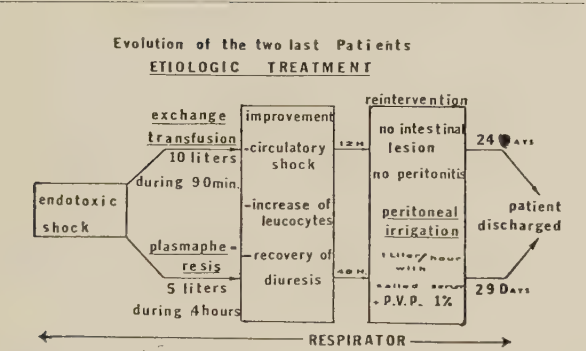


TABLE 5



was confirmed by the Limulus Lysate Test. Following exchange transfusion or plasmapheresis, the test proved negative, thus demonstrating the effectiveness of the therapy.

References

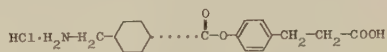
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Clinical study of antiulcer agent by double blind technique

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The double blind controlled clinical study was undertaken to investigate the usefulness of cetraxate hydrochloride for the treatment of peptic ulcer. This substance has the chemical structure of



and has been used for the treatment of pancreatitis etc. due to its antitrypsin, antiplasmin, antikallikrein and antithrombin actions. The selectively high distribution in the gastric wall and the beneficial effect to experimental ulcer were also found in animal experiment for this substance.

A series of 234 patients with gastric ulcer were investigated up to 12 weeks of medication using gefarnate as the standard drug without concomitant administration of any other antiulcer drugs.

Statistical analysis revealed that the cure rate of ulcer with lapse of time confirmed by endoscopic examination were 28, 61 and 73% after 4, 8 and 12 weeks of medication with cetraxate, while 23, 47 and 55% with gefarnate respectively. A significant difference as to the cure rate between the two drugs was detected after 8 and 12 weeks, but not after 4 weeks of treatment. The cure rate of ulcer with lapse of time was analysed further by stratifying the patients according to hospitalized vs. ambulant status as shown in Fig. 1. Namely the final cure rate was 83% in the hospitalized patients treated with cetraxate and 65% in those with gefarnate with significant difference. In the ambulant patients, however, it was 56% in the cetraxate group and 46% in the gefarnate group without significant difference. Stratified analysis revealed further, as shown in Fig. 2, that cetraxate was significantly preferable in the hospitalized patients with ulcers round to oval by shape, single by number, of first occurrence by ulcer history, shallow by depth, and tended to be preferable in the hospitalized patients with ulcers located along the lesser curvature. The same tendency as with cure rate was found in global utility rate made by the physician in charge by summarizing alterations in ulcer, symptoms, side effects, laboratory findings

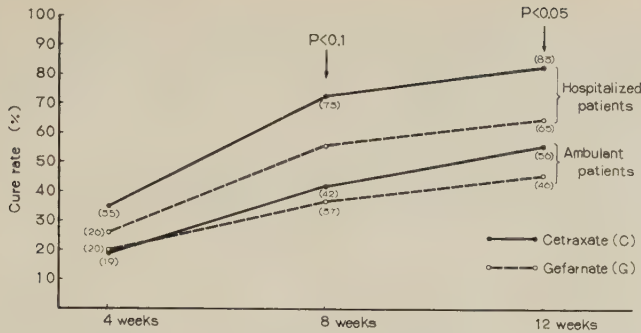


FIG. 1. Alterations in cure rate by hospitalized and ambulant status.

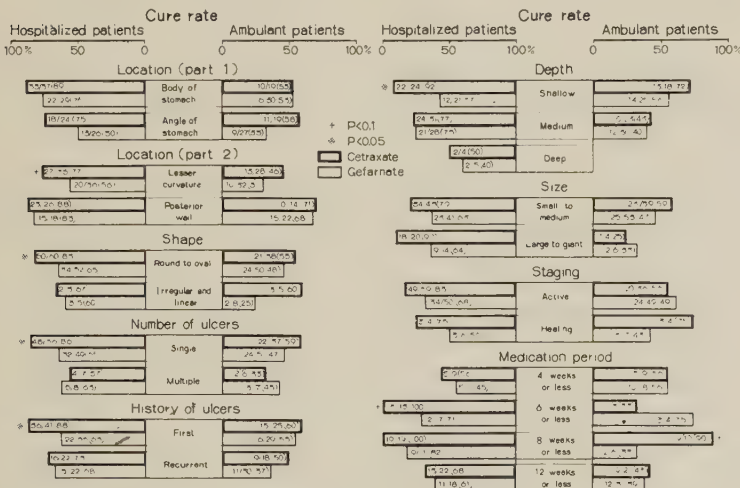


FIG. 2. Background of patients and cure rate.

and comparison with previous similar experiences. No serious side effect was encountered throughout the study. It was also interesting to note that erosive gastritis often encountered in many patients healed remarkably in cetraxate-treated group in comparison with gefarnate-treated group.

It was confirmed that cetraxate hydrochloride is useful for the treatment of peptic ulcer.

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Kinetics of upper and lower small intestinal tract bile acid concentrations after oral administration of ursodeoxycholic acid (UDCA)

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The intestinal wall being the first barrier of entrance for orally administered bile acids (BA), it is of central interest in BA metabolism and toxicity studies. Detailed information on the effects of UDCA, which has lately entered cholelitholytic trials, are lacking. We therefore undertook the following investigations. Female Wistar rats weighing 150-180 g were administered 90 mg UDCA/kg. After 15, 30, 45, 60, 90, 120 and 180 min nonsulfated BA (NSBA) and sulfated BA (SBA) were determined in the jejunal (JM) and ileal mucosa (IM) by gas-liquid chromatography. Contamination with intestinal contents was controlled by nonabsorbable radioactive markers. The JM of normal Wistar rats contains 179 μ g BA per g intestinal wet weight, main BA are cholic (CA) and chenodeoxycholic (CDCA) acids; almost half of most BA are sulfated. BA concentration in the IM with 211 μ g/g is slightly higher, the BA pattern resembles the one in the JM; sulfation rates of CDCA, deoxycholic (DCA) and lithocholic (LCA) acids are somewhat higher. These results of almost equally high BA concentrations in the JM and IM are in good agreement with late investigations by Ho; and Ahlberg *et al.*, Switz *et al.*, Angelin *et al.*, and Schalm *et al.* who pointed out that there is a substantial jejunal uptake of dihydroxy BA, according to Angelin up to 30% in man. In the rat, Sklan *et al.* found over 50% of BA absorption in the jejunum. They also report that BA absorption is accompanied by a significant increase in local mucosal BA concentrations. So these results fit well together underlining the importance of the proximal small intestine in the enterohepatic circulation (EHC) of BA. In the JM total NSBA show a significant increase after 15 min with a maximum after 45 min, a drop after 60 min and slight increases thereafter. TSABA, on the other hand, show a significant decrease from 15 to 45 min, a maximum after 60 min is again followed by a sharp decrease after 120 and 180 min. In the IM total NSBA show their maximum already after 30 min, then decreasing till 60 min with a consecutive small rise.

SBA in the IM, in contrast to the JM, show a smaller decrease till 45 min, then maintaining an even level. So NSBA show quite parallel developments in the JM and IM, while SBA display a different behaviour: in the JM, their initial drop is much sharper, their l-h-maximum is higher with another consecutive deeper decrease: SBA undulations show larger amplitudes in the JM than in the IM. In both parts of the small intestine SBA increases show much later than NSBA rises. This could be explained by the time needed for sulphate ester synthesis, and by less complete—and possible slower—EHC. More consistent levels of SBA in the IM could in part be explained by higher ileal absorption of SBA, which Low-Beer has proven for sulphated tauro—and glyco-LCA in the guinea pig. Single NSBA in the JM largely correspond to total NSBA alterations with most maxima after 45 min and consecutive decreases. Only LCA shows a later maximum—after 120 min. After 15 min already UDCA is second highest single NSBA, but never becomes highest. This is in good correspondence to the findings of Federowski *et al.* that given in equal amounts to rhesus monkeys biliary UDCA has less than half the concentration of biliary CDCA. This can be partly explained by the fact that UDCA is more readily transformed to CDCA than vice versa; it also shows a higher spillover into peripheral blood (Makino, Stiehl). Single SBA alterations in the JM reflect the behaviour of total SBA with the exception of SLCA which increases after 180 min. NSBA patterns in the IM show an earlier maximum of CA (30 min) and less pronouncedly so, CDCA (30 min), later maxima of UDCA (45 min), LCA (60 min) and DCA (120 min). SBA patterns in the IM show little changes for sulfated CA, a gradual and continuous increase in SUDCA with constantly decreased amounts of SCDCA and low concentrations of SDCA and SLCA. In the JM, increases in UDCA and CDCA appear earlier and are higher than in the IM, where the CA maximum shows earlier. While UDCA is second highest in the JM after 15 min, it reaches this rank in the IM only after 45 min. This fits well with results from Angelin *et al.* who found higher dihydroxy BA absorption than trihydroxy BA absorption in the jejunum. This behaviour of dihydroxy BA has its repercussions on EHC regulation and biliary lipid secretion possibly favouring dihydroxy BA in BA regulation activity because they appear more rapidly and more often exerting their negative feedback effect. The secondary BA DCA and LCA show their ileal maxima much later than primary BA. LCA remains low and is sulfated to a high percentage.

SUMMARY

In the JM and IM BA concentrations are of similar order of magnitude pointing to similar absorption activities. After administration of 90 mg UDCA/kg increases in total NSBA are accompanied by drops in SBA pointing to less

effective absorption or slower synthesis of SBA. Dihydroxy BA appear earlier in the JM, trihydroxy BA in the IM. UDCA never gets first rank in BA concentrations pointing to rapid metabolism to CDCA. Toxic LCA remains low and is sulphated a high percentage. Various results suggesting a jejunal short-cut of dihydroxy BA affecting regulation of EHC and biliary lipid secretion point to the necessity of further elucidating the fate of orally administered therapeutical BA in the intestinal mucosa.

Idiopathic ulcerative colitis in Istanbul

clinical review of 204 cases

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This is a report on 204 cases of Idiopathic ulcerative colitis (IUC), diagnosed and treated in the department of gastroenterology Beyoglu Insurance Hospital, Istanbul during the last 9 years. It is the largest series reported from Turkey. The clinical course, complication rate and response to therapy has been analyzed and presented.

CLINICAL MATERIAL AND METHODS

A total of 204 cases of Idiopathic Ulcerative Colitis were diagnosed and treated in the department of Gastroenterology of a 1000-bed Insurance hospital in Istanbul between 1969 and 1977.

All the patients were living in Istanbul, all had various occupations. There was no specific prevalence of disease, among the cases with respect to the geographic distribution of their origin (East, West, North, South or central Turks, Jews, Armenians, Iranians).

Distribution of patients according to age and sex at the onset and diagnosis of disease is shown on (Table 1).

The ages ranged from 3 to 76 years, average for both sexes was at 36.6 years. Likewise the onset of disease was mainly between 3rd and fourth decades. Only 11.3% of the cases were in children before age 20 and 19.1% were in those beyond age 50.

TABLE 1

Distribution of patients according to sex and age at onset and diagnostication of the disease

Age (year)	Age of onset		total
	women	men	
0-9	3	3	6
10-19	4	8	12
20-29	25	20	45
30-39	30	21	51
40-49	20	31	51
50-59	14	12	26
60-69	4	5	9
70-79	2	2	4
TOTAL	102	102	204

It will be seen from (Fig. 1), that the number of cases discovered each year between 1969 and 1977 shows an increasing trend.

The clinical diagnosis of IUC was based on criteria of histories, pathologic findings at rectosigmoidoscopy, biopsies and barium enemas. Our definition of IUC includes isolated idiopathic proctitis. Disease of the rectum and colon due to malignancies, Crohn's disease, radiation, and antibiotics as well as infections and parasites (eg. Tubercule bacillus, amoeba, salmonella, shigella, gonorrhea, syphylis Giardia lamblia) were excluded by biopsies, repeated stool exams or cultures.

Clinically, the patients presented with various signs and symptoms.

The majority of the patients, had symptoms of diarrhea, hematochezia, abdominal pain, weight loss and anxiety or depression (Table 2).

The barium enema findings are shown in Table 3. In the majority of the cases disease was either confined to left colon or rectosigmoid region. In 13 (6.4%) of the cases radiographic findings were normal, although the rectosigmoidoscopic appearances were characteristic of Idiopathic ulcerative colitis (IUC).

TABLE 2

Clinical date of 204 patients at time of diagnosis of ulcerative colitis

<i>Symptoms of finding</i>	<i>Number of patients</i>			
	<i>women</i>	<i>men</i>	<i>total</i>	<i>percent</i>
Diarrhea	80	89	169	82.8
Hematochezia	95	86	181	88.7
Abdominal pain	81	76	157	76.9
Weight loss	60	62	122	59.8
Tenesmus	42	44	86	42.1
Fever	22	33	55	26.9
Constipation	19	10	29	14.2
Anxiety or depression	87	98	185	90.6
Joint pains	37	29	66	32.5
Milk allergy	31	38	69	33.8
Generalize itching	3	1	4	2.0
Family history			9	4.4

TABLE 3

Colonic involvement by barium enema during reference period in hospital

	<i>Number of patients</i>			
	<i>women</i>	<i>men</i>	<i>total</i>	<i>percent</i>
Entire colon	10	11	21	10.3
Left side on transverse	1	1	2	1.0
Left side only LC	52	36	88	43.1
Rectosigmoiditis RS	41	39	80	39.2
Normal colon reported	12	1	13	6.4

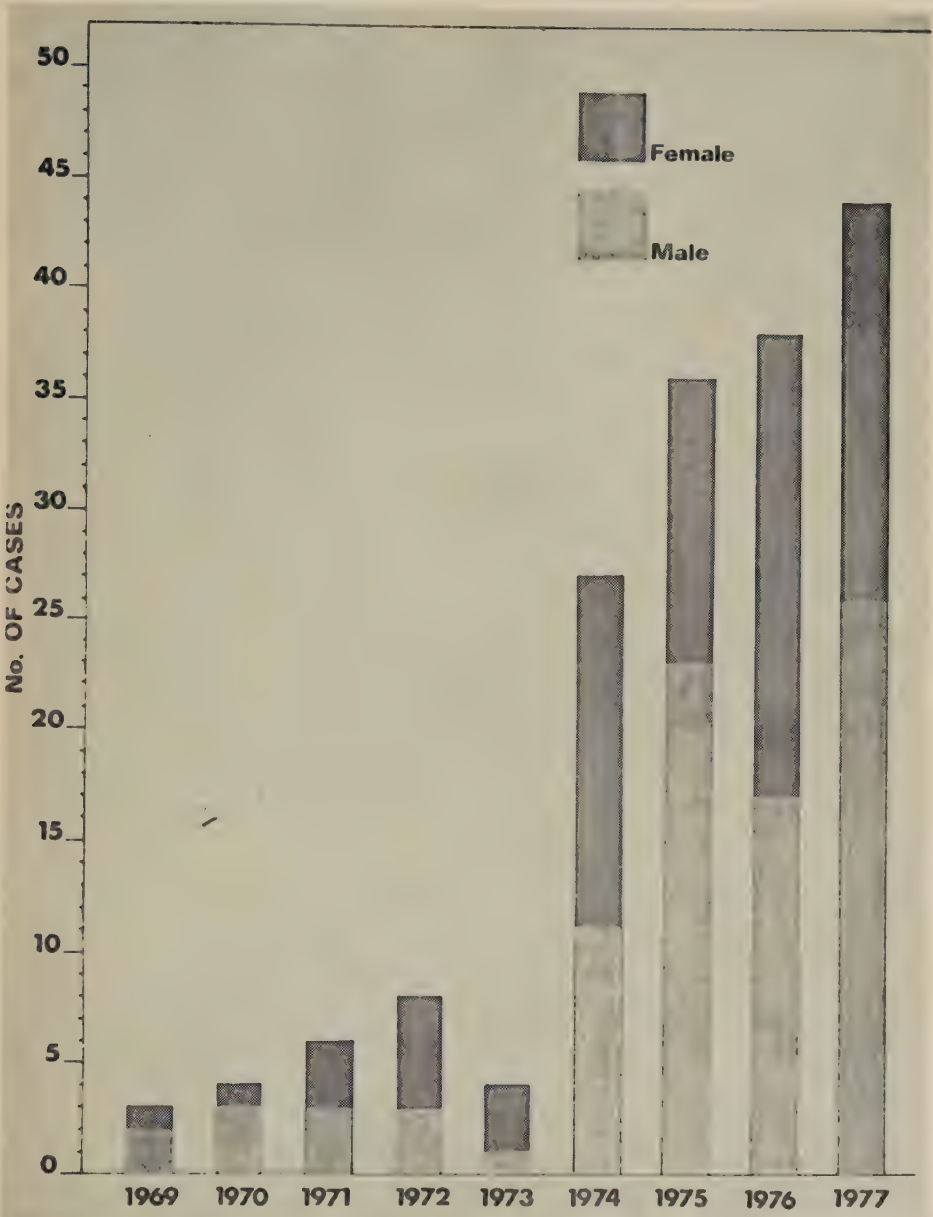


FIGURE: Newly diagnosed cases of ulcerative colitis 1969 through 1977.

FIG. 1. Ulcerative colitis and proctitis new cases (170 Patients).

The stages of disease were graded as mild, moderate and severe as described by Truelove and Witts (2).

(Table 4) gives the clinical severity and its distribution according to sex. Majority of the patients in both sexes had mild form of disease (74%).

This may in part be due to earlier diagnosis of cases but, as shown on Table 5, the majority of these patients had the disease 1 to 5 years at the time of presentation, suggesting that mild form of disease is nevertheless more prevalent and may present as such after a somewhat chronic course.

TABLE 4

Composition of series by sex and clinical severity

<i>Form</i>	<i>Women</i> <i>No. of cases</i> %	<i>Men</i> <i>No. of cases</i> %	<i>Total</i> <i>No. of cases</i> %
Mild	73 (71.6)	78 (76.5)	151 (74.0)
Moderate	14 (13.7)	9 (8.8)	23 (11.3)
Severe	15 (14.7)	15 (14.7)	30 (14.7)
TOTALS	102	102	204

TABLE 5

Duration of Diseases at time of presentation

<i>Years</i>	<i>Men</i> <i>No. of cases</i>	<i>Women</i> <i>No. of cases</i>	<i>Total</i> <i>No. of cases</i>	%
Less than 12 months	17	14	31	15.19
1- 2 Year.	16	14	30	14.70
2- 3 yr.	17	17	34	16.66
3- 4 yr.	10	12	22	10.78
4- 5 yr.	5	14	19	9.31
5- 6 yr.	6	9	15	7.35
6- 7 yr.	3	2	5	2.45
7- 8 yr.	6	4	10	4.90
8- 9 yr.	2	4	6	2.94
9-10 yr.	2	0	2	1.08
10-15 yr.	7	4	11	5.39
15-20 yr.	3	2	5	2.45
> 20 yr.	3	4	7	3.43
Unknown	5	2	7	3.43

The clinical course of the disease and response to therapy appear to parallel the severity of disease (Table 6). Thus the acute fulminating cases (8 patients) died in the course of one or more year. Where as those with single attack are

TABLE 6

Course of disease (204 patients)

<i>Group</i>	<i>Course of disease</i>	<i>Number of patients and Percent of total</i>	
I	<i>Acute fulminating</i>		
	a) A single attack going on to death in less than one year	5	3.9
	b) Going to death in more than one year	3	
II	<i>Chronic intermittent</i>		
	More than one attack with at least several weeks complete freedom between attacks	28	13.7
III	<i>Chronic continues</i>		
	Continuous symptoms for more than one years	11	5.4
IV	<i>Single attack</i>		
	Followed by complete freedom	151	74.0
V	<i>Unclassified</i>		
	Follow up failed	4	2.0
		204	

now well and symptoms free (74%). 13.7% of the patients had chronic course with intermittent exacerbations, 5.4% had continuous chronic course for more than one year.

Stated differently 196 (96%) of the patients are presently alive, having received medical or surgical treatment. Only 8 (3.9%) are dead. Despite medical, surgical therapy for IUC (Table 7).

TABLE 7

Fate of traced patients (204 patients)

<i>Fate of patients</i>	<i>Number of patients</i>	<i>%</i>
<i>Living</i>	196	96
Of ulcerative colitis without operation	188	
Of ulcerative colitis with operation	8	
<i>Dead</i>	8	3.9
Of ulcerative colitis without operation	5	
Of ulcerative colitis with operation	2	
Of other cause without operation	1	

The variety of related problems seen in our group of 204 cases of IUC are listed in Table 8.

Most common among these were fissure in ano and hemorrhoids, pseudo-polyposis, anemia, aphthous stomatitis and liver disease. Of note is the low occurrence rate, toxic-megacolon (1.0%), and cancer 1.0%).

Among the associated disorders psychiatric and peptic ulcer disease and nephrolithiasis were most common, in incidence perhaps more frequently seen with IUC than in general populations (Table 9).

TREATMENT AND RESULT

All patients received medical management consisting mainly of high-calories, low-residue diet, vitamins, rest, steroid sulfa. No psychotherapy was given. In 8 patients with acute fulminating course azathioprin was given in addition to steroids (Table 10).

TABLE 8

Complication of ulcerative colitis (204 patients)

	No. of Patients	%
<i>Rectal</i>		
Hemorrhoids	19	9.3
Fissure in ano	22	10.8
Fistula in ano	6	2.9
Perianal or ischiorectal abscess	1	0.5
Prolapsus of recti	1	0.5
<i>Colonic</i>		
Toxic Megacolon	2	1.0
Pseudo-Polyposis	23	11.3
Single Polyp	2	1.0
Carcinoma	2	1.0
Massive Hemorrhage	3	1.5
Perforation	1	0.5
<i>General</i>		
Anemia	90	44.1
Aphthous Stomatitis	28	13.7
Liver Disease (Hepatitis, etc.)	14	6.8
Pericholangitis	1	0.5
Skin Dis. and Erythema Nodosum	11	5.4
Clubbing of fingers	16	7.8
Growth Retardation	9	4.4
Mental Disturbance	2	1.0
Iritis Uveitis	1	0.5
Ankylosing Spondylarthros	1	0.5

TABLE 9

Associated diseases in our group of 204 patients with ulcerative colitis

<i>Diseases</i>	<i>No. of Patients</i>	<i>Percent of 204 Patients</i>
Peptic ulcer	21	10.2
Psychiatric illness	30	14.7
Urinary calculus	15	7.3
Hayfever	10	4.9
Diabetes mellitus	5	2.4
Chronic bronchitis	4	2.0
Nodular goiter	4	2.0
Non-toxic goiter	3	1.5
Thyrotoxicosis	1	0.5
Gallstones	3	1.5
Cerebrovascular accident	3	1.5
Eczema	2	1.0
Gastrointestinal hemorrhage* (Severe Hematemesis-Melena)	2	1.0
Obesity	2	1.0
Asthma	1	0.5
Psoriasis	1	0.5
Epilepsy	1	0.5
Parkinson's disease	1	0.5
Thrombophlebitis	1	0.5
Diverticulosis of colon	1	0.5

* Nothing found in operation

TABLE 10

Therapeutic regimens used for patients with ulcerative colitis

<i>Medication*</i>	<i>No. of patients</i>	<i>%</i>
Sulfa preparation (orally)	173	84.8
Steroid suppositories	47	23.0
Steroid enema	107	52.4
Systemic steroids	28	13.7
Acth (I. V)	7	3.4
Azathioprine (imurek)	8	3.9

* Often more than one agent was used.

Ten patients had surgery to supplement medical therapy. This consisted of total colectomy in nine, segmental resection in one patient. Two of the nine underwent emergency surgeries and died postoperatively. The rest had elective

surgery (Table 11). The indications for elective surgery were: resistance to medical management in 5, cancer in 2 and polyposis of sigmoid in one. Results were good in all patients who underwent elective surgery.

TABLE 11

Surgical treatment and results

<i>Operation</i>	<i>Cases</i>	<i>%</i>	<i>Result</i>
I. <i>Total colectomy</i>	9	4.4	
A) <i>Emergency</i>	2	1.0	EKS
B) <i>Elective</i>	7	3.4	
a) <i>Resistant to medical treatment</i>	5	2.4	Good
b) <i>Cancer of sigmoid colon</i>	2	1.0	Good
II. <i>Segmental resection</i>	1	0.5	Good
(Polyposis of sigmoid)			

DISCUSSION

The term "Simple Ulcerative Colitis" first appeared in literature in 1875. According to Goliger *et al.* (3) noncontagious types of diarrhea associated with colonic inflammation were recognized as early as 300 AD. So far all the published cases of ulcerative colitis in literature are from the U.S., U.K. and Northern Europe (specially Scandinavia).

Epidemiologic studies on the incidence of Idiopathic ulcerative colitis have been published in the United States, England, Norway, Sweden, Denmark, Finland, Belgium, New Zealand, Poland, Czechoslovakia and Israel (4, 5, 6, 7, 8, 9, 10, 11, 12, 13, 14, 15, 16).

Idiopathic Ulcerative Colitis (IUC) in Turkey has been considered a rarity by many Turkish authorities (17). This has not been our experience. No part of the world is immune, no racial group either.

Aktan *et al.* (17) recently reviewed 60 patients from Ankara.

In Istanbul from 1969 to 1977, we have seen 204 cases of IUC. This is the largest series published in Turkey.

One wonders why fewer of these cases are seen in the tropics and why they are more common in the Northern and Southern temperate zones. The reason for this may in part be related to the awareness of the condition, diagnostic facilities or presence of trained gastroenterologists.

The trend of increasing number of cases in our series during the recent years, may reflect this aspect rather than true increase in the incidence of IUC.

Unfortunately IUC is not a reportable disease. Therefore, the true incidence of this entity is hard to determine.

The extent of colonic involvement affected by IUC is a major determinant of the clinical course of the disease.

The data of various authors are not easily comparable. The classification of "distal" disease (rectosigmoiditis) varies. In some series, patients with IUC are unselected, whereas others consist of more severe cases. Comparing the results with those of other series we find that the incidence of rectosigmoiditis in Istanbul (45.6%) is nearest to that of Nefzger and Acheson (18) (47%), and Aktan *et al.* (17) (40%). Rectosigmoiditis is seen mostly in individuals in the third and fourth decades. Slightly more women than men are affected. This is confirmed in our series also (53 women and 40 men). Rectosigmoiditis commonly presented as the "mild" form of disease. There was no mortality in this group, in our series.

Mortality in our entire series was 3.9%. Death occurred mainly in patients with "severe" form of disease, involving the entire colon. Entire colon was involved in 10.3% of the cases in this series.

The course of the disease in our series was estimated as mild in 74%, moderate in 11.3% and severe in 14.7%, according to defined criteria (2). The corresponding figures for a series of 399 cases from Edinburgh (21) 45%, 25%, and 30%; for 645 cases from Czechoslovakia 52.1%, 20.9% and 27%; for 60 cases from Ankara (17) 43.3%, 36.6% and 20%; for 219 cases from Tel-Aviv 63.3%, 22.4%, and 14.1% (Table 12).

In none of these three series were the criteria for severity defined in the same time. Therefore the data are not comparable. It is nevertheless, noteworthy that the disease was considered to be mild in about half of the cases in all series.

The late complication of carcinoma of the colon, occurring in IUC in our series was (1.0%). This is also true for reports from Israel (15) (0.6%), Czechoslovakia (14) (0.5%) and from Ankara (17) 0%). This complication rate is lower than in reports from Anglo-Saxon countries.

TABLE 12

Course of the disease of ulcerative colitis comparative data

Reference	Location	No. of cases	Mild %	Moderate %	Severe %
Jalan <i>et al.</i> (21)	Edinburgh	399	45	25	30
Nedbal and Maratka (14)	Prague	645	52.1	20.9	27
Aktan <i>et al.</i> (17)	Ankara	60	43.3	36.6	20
Gilat <i>et al.</i> (15)	Tel-Aviv	219	63.3	22.4	14.1
Present Series	Istanbul	204	74	11.3	14.7

SUMMARY

Clinical review of 204 cases of IUC seen in an Insurance Hospital in Istanbul during the last 9 years is presented. The criteria of diagnosis were based on history, rectosigmoidoscopy biopsy and barium enema. The mortality for the whole series was 3.9%. Deaths occurred in 8 patients who had "severe" form of disease involving the entire colon and presented a fulminating course. One additional death was due to an accident.

Treatment consisted of high-protein, low-residue diet, vitamins, rest, sulfa and steroids. Azathioprin (Imurek) in addition, was used in fulminating cases.

Surgery supplemented medical therapy in 10 cases. Indications were resistance to medical management, cancer and polyposis.

Only 2 cases (1.0%) developed cancer. During the follow-up these too were operated.

IUC is not a rare entity, with awareness and use of appropriate diagnostic facilities more cases are being discovered in recent years.

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Endotoxemia and complement activation in acute pancreatitis

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In 1973 Woodruff *et al.* detected endotoxemia in a patient with acute pancreatitis. Thus, Fine (1975) incriminated endotoxin as of causative for a number of extrapancreatic symptoms, such as clotting disorders, vascular collapse and intestinal hemorrhagic ulcerations. It was thought that these substances cross the gut wall from the transverse colon. Since endotoxins may also activate the complement system by the alternate pathway (Götze and Müller-Eberhard, 1971) experimental data are of interest which indicate that an activated complement system contributes significantly to the pathogenesis of acute pancreatitis (Seelig and Seelig, 1975). In order to investigate whether endotoxemia is frequent in acute pancreatitis and whether the complement system is concomitantly involved, 33 patients with acute pancreatitis were investigated by means of the Limulus Gelation Test (Liehr *et al.*, 1976) for detection of endotoxemia, and by evaluation of the complement hemolytic activity (CH_{50}) and the complement factor 3 (C_3) plasma concentrations (for details see Seelig and Seelig, 1975). The data obtained are summarized in Table 1, Figs 1 and 2. The results indicate that endotoxemia is a frequent event in acute pancreatitis and that

TABLE 1

Frequency of endotoxemia in acute pancreatitis and frequency of severe extrapancreatic disorders in correlation to endotoxemia

Patients (n = 33)	Limulus-Gelation-Test	
	positive 22 (66%)	negative 11 (33%)
Renal failure	5	1
DIC	10	3
Pulmonary disorders	2	0
Need for intensive care	15	3
Death	9	0

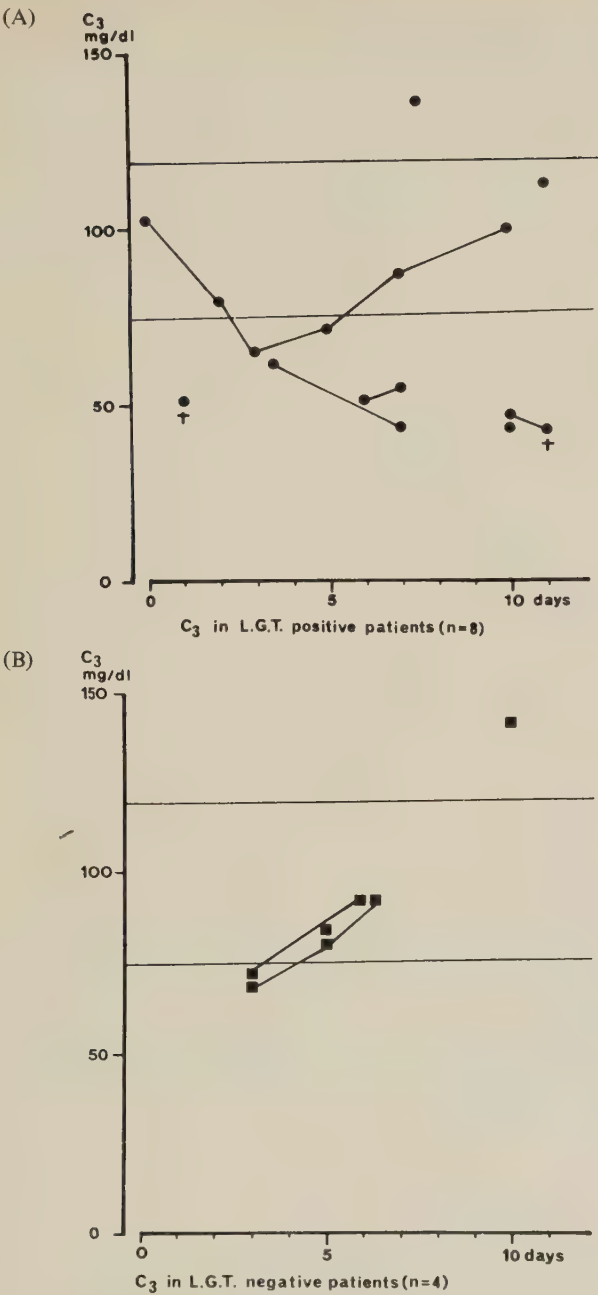


FIG. 1. Plasma concentrations of complement factor 3 (C₃) in endotoxin positive (A) and endotoxin negative (B) patients in correlation to the onset of the acute attack.

severe extrahepatic events preferentially occur once endotoxemia is present. It is remarkable that only endotoxin positive patients died. Most marked decreases of C_3 were observed in endotoxin positive patients (Fig. 1a), and two patients died, in whom no normalisation happened within ten days. In endotoxin negative patients no such decreases of C_3 were present (Fig. 1b). The data on CH_{50} indicate that a decrease of values down to 50% was associated with death of the patients (Fig. 2). The conclusion is that endotoxemia contributes signi-

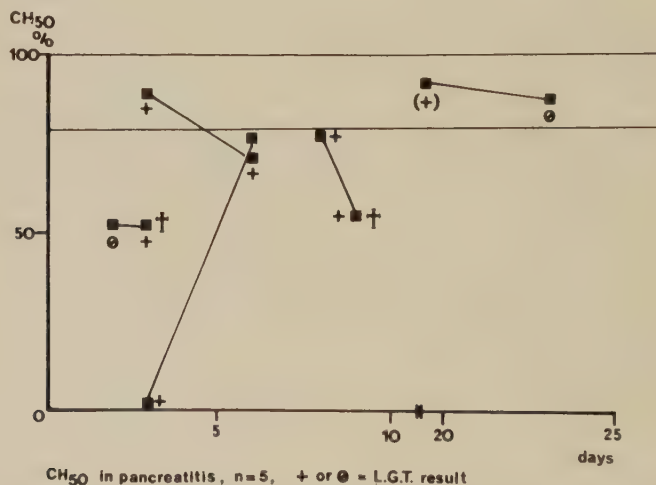


FIG. 2. Complement hemolytic activity (CH_{50}) in acute pancreatitis; ○ and + indicate result of the Limulus Gelation Test (L.G.T.).

ficantly to severe courses of acute pancreatitis, and that the complement system predominantly is involved in the presence of systemic endotoxemia. A thus activated complement system, suggestively, may lead to additional pancreatic acinous necrosis by its cytolytic activity as proposed recently (Seelig and Seelig, 1975). It seems reasonable that both events form a vicious circle responsible for death in acute pancreatitis.

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Effect of physical exercise on basic acid output in men with chronic duodenal ulcer disease

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In 12 men aged 22-57 years (mean 37.7 years) with chronic duodenal ulcer disease of 6.6 years mean duration and active duodenal bulb ulcer there were carried out the investigations of basic acid output in sitting position in rest, during physical exercise of one-hour duration. The men performed physical exercise on bicycle ergometer (Monark) with the load giving 50 per cent increase in heart rate in relation to the maximal heart rate.

During physical exercise BAO increased from 5.42 ± 4.72 to 9.55 ± 6.91 mEq CH₁, gastric hydrochloric acid concentration increased from 57.0 ± 24.2 to 75.4 ± 17.8 mEq/l and gastric acid volume increased from 88.1 ± 42.7 to 115.4 ± 58.9 ml/hr; these changes were statistically significant ($p < 0.01$). Moreover, during physical exercise there were found significant increases in gastric juice concentrations of potassium, sodium chloride, magnesium and calcium.

During one-hour recovery there were found the decreases in gastric juice volume and hydrochloric acid (HCl) concentration and in BAO in relation to the values obtained during exercise ($p < 0.05$) and in relation to the initial value ($0 < 0.05$). Moreover, during recovery the decrease in gastric electrolyte secretion was observed.

The studies performed on 15 healthy men in the same conditions revealed the decrease in gastric juice volume and HCl concentration, the decrease in BAO and electrolyte content during exercise and more still during recovery.

Effects of a mixed cupric complex with L-aminoacids (copper-tryptophane-phenilalanine) on rat gastric acid secretion stimulated by pentagastrin, histamine and bethanechol

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Antiarthritic and antiulcer activity of copper chelates was first described by Sorenson (10). We have shown afterwards (1, 6, 7) that copper complexes with aminoacids, in addition to preventing ulcer formation, were also able to inhibit gastric acid secretion in Shay rat. This antisecretory effect was related to employed doses and, at equal copper amounts, was directly proportional to the liposoluble complexes concentrations in solution, at certain intragastric pH values. The latter was obtained from stability constants (concerning cupric ion equilibrium with employed ligants) and from distribution of the species in function of pH values. Calculation programs Acba (2), Miniquad (9) and Disdi (5) were used. The mechanism of action of these compounds on gastric acid secretion is still unknown. In this study we investigated the action of a mixed copper (II) complex with L-aminoacids, copper-tryptophane-phenilalanine (Cu-Tryp-Phe), on gastric acid secretion stimulated by pentagastrin, histamine and bethanechol.

METHODS

The experiments were performed on fasted male C.D. rats (b.w. 250 ± 10 g), under urethane anesthesia (1.35 g/Kg i.m.), and the stomach in situ perfusion method described by Ghosh and Schild as modified by Barrett *et al.*, was used (3). Ten minute fractions of perfusion fluid were collected and the total acidity was determined by titration with NaOH 0.01 N using phenolphthalein as indicator. Gastric acid secretion was stimulated by i.v. administration of pentagastrin (2.5 μ g/Kg), histamine dihydrochloride (500 μ g/Kg) or bethanechol chloride (150 μ g/Kg). Acid output induced by each of these agents was measured before and 90 minutes after Cu-Tryp-Phe administration (45 mg/Kg in 1 ml of Tween

80–25%, given intragastrically after stopping saline perfusion) and was expressed as the net total acid output increase over 1 h period ($\Delta \mu\text{Eq H}^+/\text{h}$). Percentage inhibition was then calculated.

RESULTS

In basal conditions the net acid output increase ($M \pm SD$) has been 23.68 ± 11.23 after pentagastrin, 16.54 ± 1.91 after histamine and 28.78 ± 5.14 after bethanechol. Intragastric administration of Cu-Tryp-Phe reduced these values respectively to 2.98 ± 1.30 (inhibition 87%), 2.90 ± 0.82 (inhibition 82.5%) and 5.30 ± 1.65 (inhibition 82%). All data were significant ($0 < 0.01$).

TABLE 1

Action of Cu-Tryp-Phe on stimulated gastric acid secretion in rats

<i>Compounds</i>	<i>$\Delta \mu\text{Eq H}^+/\text{h}^*$</i>	<i>% Inhibition</i>
Pentagastrin	23.68 ± 11.23	
Pentagastrin + Cu-Tryp-Phe	$2.98 \pm 1.30^{**}$	87
Histamine	16.54 ± 1.91	
Histamine + Cu-Tryp-Phe	$2.90 \pm 0.82^{**}$	82.5
Bethanechol	28.78 ± 5.14	
Bethanechol + Cu-Tryp-Phe	$5.30 \pm 1.65^{**}$	82

* $\mu\text{Eq H}^+ (M \pm SD)$ secreted above the basal value

** $p < 0.01$

Our results show that Cu-Tryp-Phe causes a strong inhibition on rat gastric acid secretion stimulated by pentagastrin, histamine and bethanechol. Previous studies demonstrated also that cupric ions influence the biosynthesis of prostaglandins (4). These substances, as known, inhibit gastric acid secretion both in animals and humans and, consequently, could play a very important role on neuro-hormonal control of gastric acid secretion (8). Therefore it is quite possible that antiulcer and antisecretory activity of copper (II) complexes be mediated, at least partially, by prostaglandins.

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HLA Antigens in coeliacs and controls in the West of Ireland

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The prevalence of coeliac disease is high in the West of Ireland (1). This is so in cases presenting in childhood and in adult life. Amongst 52 University technicians, 2 have coeliac disease. Amongst 420 nurses, 1 has the disorder and amongst 350 medical students and 52 Professors, 2 and 1 respectively have the disease. Of 55,183 children born in County Galway, between 1960 and 1976, 96 have developed the disorder to date (2). This indicates at least 1 in 575 children develop the disorder. Approximately two-thirds of coeliacs develop their symptoms before the age of 16, so the total prevalence in the population is 1 in 350, when allowance is made for those presenting after the age of 16.

Reasons for the high incidence are not evident, but the high incidence must, at least in part, be due to genetic factors.

At the time the study was started, the only genetic marker related to coeliac disease was HLA B8 (3, 4). Because of this, a study of HLA antigens of A and B series was carried out.

One hundred and seventy three patients with biopsy proven coeliac disease were studied and 105 controls. The controls were all third generation West of Ireland stock and were excluded unless their serum iron and haemoglobin were normal. They were also excluded if a first degree relative was known to have coeliac disease. The results are indicated in Table 1.

TABLE 1
HLA antigens in the West of Ireland

	A1	A2	A3	A9	A10	A11	A28	A29	AW30	A31	A32
Controls % (105)	48	39	16	17	18	21	4	2	3	0	5
Coeliacs %	63	41	21	12	9	10	1	3	—	4	9
	B5	B7	B8	B12	B13	B14	B18	B27	BW15	B16	B17
Controls %	4	34	42	29	1	13	6	8	4	6	5
Coeliacs %	7	23	77	30	—	13	4	3	2	2	2
	B21	B32	B35	B37	B40						
	2	2	7	—	6						
	1	—	4	6	5						

The incidence of HLA B8 in coeliac patients is as found in other studies in England (3) and U.S.A. (4). It is higher than in Austria and Germany (5). The incidence of HLA B8 in the controls is much higher than elsewhere in Europe, where England 30% (3), Austria and Germany 18% (5), Italy and France 13% and 11% (6) are average figures.

The high incidence of HLA 8 in the West of Ireland may perhaps be explained by considering the history of Ireland. In 800 A.D., the population was Celtic in origin. Since that time, successive invasions and plantations have tended to move the original Celtic stock to the West of Ireland. This contention is supported by the finding of an incidence of 35% for HLA 8 in the Dublin area of the Country (7).

The high prevalence of HLA 8 in the West of Ireland indicates that the genetic background against which coeliac disease occurs is common in the West of Ireland, and at least partly explains high prevalence of the disorder.

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Simultaneous study of gastric secretion and antral motility-observation on the action of two synthetic anticholinergics

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A test that aims to evaluate gastric function through two parameters observed simultaneously is systematized: gastric secretion of HCl and antral contractions. Eighteen patients suffering from duodenal ulcer were studied by means of three coupled polyvinyl catheters, the distal extremity of which was located fluoroscopically in the antrum. After 60 minutes of study in basal conditions, normal saline (6 cases) or 20 mg of N-butyl hyoscine (6 cases) or 5 mg iso-indoline (etomidoline) (6 cases) was administered intravenously. The acid secretion, aspirated manually and divided into aliquots of 10 min, was titrated with NaOH 0.1 N.

The variations in pressure registered by a Hewlett-Packard physiograph were analysed by counting the number of antral contractions and measuring the average amplitude of two pressure channels, expressed in cm. H₂O, for periods of 10 min. The results statistically analysed (which will be presented) showed that in comparison with the basal period: (1) the normal saline did not exercise any effect on the parameters under consideration; (2) neither the N-butyl hyoscine nor the iso-indoline (etomidoline) exercised any action on the gastric secretion; (3) the N-butyl hyoscine reduced both the frequency of the antral contractions ($p < 0.05$) and their amplitude ($p < 0.05$), being this action more intense in the first 30 min after administration; (4) the iso-indoline (etomidoline) did not produce any statistically appreciable effect on the frequency of the antral contractions ($p < 0.05$); however, the drug had considerable effect on the amplitude, reducing it ($p < 0.05$) in the same way as N-butyl hyoscine, the effect being particularly intense in the first 30 min after the injection. The authors conclude that the method permits the evaluation of gastric function principally in the comparative study of the action of drugs. On the other hand, comparison between N-butyl hyoscine and iso-indoline (etomidoline), in the doses indicated, revealed that the first acts by reducing the frequency and amplitude of the antral contractions, while the second acts predominantly by reducing the amplitude without effect on the physiological frequency of the contractions.

The value of an elemental diet in the management of complicated Crohn's disease

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Many patients with Crohn's disease have severe malabsorption and nutritional deficiency, chronic fistulae, local infections and perianal ulceration, and diarrhoea associated with malabsorption of fat, water, electrolytes, or bile acids. The value of using an elemental diet in a group of patients with complicated Crohn's disease has been assessed. Elemental diets contain a protein source, carbohydrate, fat, trace elements, vitamins and electrolytes. They provide dietary components in a readily assimilable form with only minimal digestion requirements, and with minimal residue (Russell, 1975; Russell, 1978). The elemental diet Vivonex was administered either orally or by nasogastric tube. In all patients nutritional status was maintained or improved with elemental diet therapy. In six patients with severe anal ulceration and fissuring, substantial improvement or complete healing of the local lesion was achieved within 2-3 weeks of starting treatment with an elemental diet. In two patients with sub-acute ileal obstruction, Vivonex allowed the obstruction to subside without recourse to surgery. In six patients with severe watery diarrhoea associated with bile acid malabsorption, an improvement in the severity and urgency of the diarrhoea was observed. This was associated with a significant reduction of faecal bile acid excretion from 6.37 ± 1.64 (SEM) mmol/24hr to 2.70 ± 1.12 (SEM) mmol/24hr ($0 < 0.05$).

The elemental diet Vivonex may thus be of value in improving the nutritional status of patients with Crohn's disease, clinically improving diarrhoea associated with bile acid malabsorption, and may allow healing of local anal infections and perianal ulceration by virtue of its low residue nature.

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Humoral and cellular immunity in inflammatory bowel disease

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There is considerable evidence for the presence of pathological immune-phenomena in inflammatory bowel disease (IBD) (1). This includes the occurrence of antibodies and cytotoxic lymphocyte reactivity to colonic epithelium (2-4), immune complexes (5, 6), complement activation (7) and an increase in immunoglobulin producing cells in the intestinal mucosa (8). Investigations of lymphocyte reactivity to mitogens did not reveal consistent results (9, 10). Recently, new techniques have been introduced, e.g. determination of helper-cell counts (11) and evaluation of suppressor cell activity (12), techniques that could add further information in respect to the immunological aspects of the pathogenesis of IBD.

15 patients with IBD (6 with Crohn's disease, 5 with ulcerative colitis, and 4 with no definite diagnosis at the time of investigation) entered the study. Diagnosis was established by the following criteria: history, physical examination, laboratory tests, X-ray of the small and large intestine, proctoscopy and/or colonoscopy, histological features of bioptical specimens. All patients were free of medication for at least 3 weeks prior to the investigation. All but one patients were in an inactive stage of the disease.

The following immunological investigations were performed (due to technical reasons these investigations could not be done uniformly in all 15 patients):

1. Serum-immunoglobulin levels (immunodiffusion).
2. Antibodies to nutritional antigens using a double antibody technique and ³H-labelled ovalbumin, bovine serum albumin, lactoglobulin, and gliadin (Menzel *et al.*, in preparation).
3. Immune complexes using a solid phase Clq-assay (13).
4. T-cell counts (E-rosette technique).
5. Number of lymphocytes positive for IgM-Fc-receptors using IgM-coated bovine erythrocytes (this technique possibly enables to count "helper"-cells, although very recent evidence is contradictory-14).
6. Suppressor cell activity using ConA-induced suppressor cells and ConA-transformation of allogeneic responder cells.
7. Lymphocyte transformation to PHA, PWN, and ConA.
- 12 Healthy individuals (age- and sex matched) served as controls.

RESULTS AND DISCUSSION

With single exceptions, immunoglobulin-levels were within normal range. In 5 patients antibodies to one or more of the nutritional antigens could be detected. The presence of circulating immune complexes was demonstrated in 8 of 10 patients. With 2 exceptions (47%, 56%) T-cells were within normal range (60-90%). In the 6 investigated patients, lymphocytes positive for IgM-Fc were also within normal range (30-60%) as was the suppressor-cell activity (in general 30-50% inhibition of Con-A-transformation, but occasionally patients as well as controls in a lower range, 1 patient showed enhancement as did 1 control). Lymphocyte transformation to PHA was significantly depressed in IBD-patients ($0 < 0.001$); similar observations were done with ConA ($p < 0.01$), but not with PWM. No differences between Crohn's disease and ulcerative colitis could be observed.

Our results indicate that pathological immunological phenomena can be observed in patients with IBD; the most striking observations were the occurrence of immune-complexes and the depression of lymphocyte reactivity to T-cell-mitogens. Suppressor cell-activity did not seem to be altered. However, further investigations in a larger group of patients will be necessary to fully determine the immunological abnormalities and their potential pathogenetic influence in IBD.

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Some problems of pathogenesis of ventricle motor function disturbance during ulcerous disease

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Profound transformations of muscular apparatus on morphological, histological and histochemical levels¹⁻² have significant importance in pathogenesis of disturbance of motoric-evacuatoric function of ventricle during ulcerous disease. The present report deals with investigation results of mechanoactive proteins of ventricle muscles with patients having ulcerous disease of duodenum without motoric function disturbance (control group) and with expressed motoric insufficiency due to pyloroduodenal area stenosis (main group). 200 patients were studied. Besides general clinical examinations electrogastrography and gastrofibroscopy were made. In 114 cases resected part of the ventricle was studied, 52 had expressed motoric-evacuatoric insufficiency. Investigations showed that cases with expressed motoric insufficiency are characterized by reduction of myofibrillar protein content at the expense of actomyosine complex reduction; contractile capacity of glycerinized muscle fibres (GMF) in isotope regime, tensile force of contracted GMF are reduced. ATF-ased activity, heat resistance and characteristic viscosity of actomyosine are reduced, complex-forming function of actin and myosine is disturbed. Certain correlation of fractions marked in the control group (one fraction of heavy meromyosine, one fraction of actin, two chains of light meromyosine) is disturbed at the expense of active fraction output decrease and sometimes—fraction of light meromyosine. Significant changings established in subcellular macromolecular formations determine disturbances of ventricle contractile capacity. These changings correlate with morphological, electrophysiological and clinical indices.

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Pathologist and gastroenterologist not yet married to each other

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Ottenjann (1977) quotes—not completely free of malignant delight—a crisis of pathology particularly concerning the pathohistology of gastroenterology. The expansion of bioptic diagnosis has “effected” pathologists unexpectedly. We are entirely in agreement with him (see Fig. 1). In our material we have a clear view over more than 12000 patients with nearly about 60000 bioptic samples. That is our background which qualifies us for making a critical reflection on the working situation of pathologists and gastroenterologists. Certainly we are only informed about the conditions in our own country. 21 clinics of internal medicine or surgery and two practitioners are sending to our institute biopsies

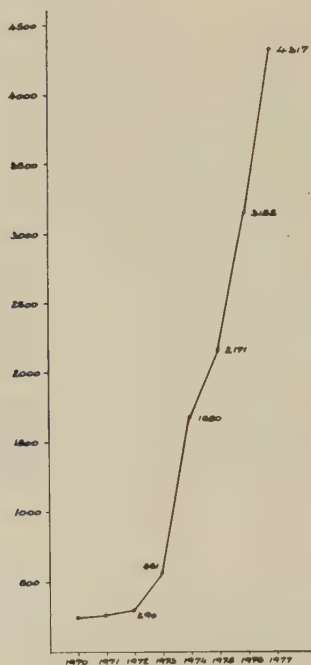


FIG. 1

of the gastrointestinal tract (Fig. 2). And in this way also a permanent crisis in gastroenterology begins. This phenomenon explains itself by the fact that only in 10 of the 23 sites for gastroenterologic examination doctors are employed with practical experience. Therefore a good cooperation with pathologists is possible (see arrows in Fig. 2). The dotted lines in Fig. 2 symbolize an actual



FIG. 2

“missing link”. In these hospitals gastroscopy is practised by diverse young doctors who are completing their training as an assistant physician. In more than 60% we get uninformative clinical data. Once more we quote Ottenjann verbatim: “paradoxic situations opened up when gastroenterologists had to give to pathologists some useful hints how to classify histological structures relating to interpretation and nosology”. We dare say this is not a paradoxic situation in reality, this is a good cooperation. Let us make an important statement from the pathologist’s point of view: structure is unseparably linked with function. That means that the histomorphologist is compelled to draw conclusions to the disfunction of gastrointestinal mucosa by the help of a snapshot in his microscopic slides. Without exact declarations of clinical symptoms and the course of disease pathologists are not able to acquire a knowledge in this field. As shown in Fig. 3 with all due modesty, the pathologist needs the answer of these basic questions. Only in this way a nosologic assignment of morphological structures is possible.

A further essential problem for a good teamwork is a distinct common terminology. No difficulties present histologic diagnosis and communication with the clinician in inflammatory processes. A first factor of uncertainty is the term

1. Age and sex
2. Duration and nature of complaints
3. Loss of weight
4. X-ray examination
5. Analysis of gastric juice (pH etc.)
6. Pernicious anemia
7. Gastroscopic findings

<ol style="list-style-type: none"> a) Normal findings b) Discoloration c) Rubor d) Swelling e) Atrophy (location) f) Hypertrophy (location) g) Abnormal plicae h) Increased vulnerability i) Abnormal vascularity j) Hemorrhage 	<ol style="list-style-type: none"> k) Erosion l) Peptic ulcer (size, form, location) m) Polyps (size, form, location) n) Tumor (size, form, location) o) Stenosis (location, dimension) p) Diaphragmatic hernia q) Reflux r) Pylorus s) Bulbus duodeni t) Papilla vateri
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8. Congo red
9. Location of biopsy and number
10. Summary of gastroscopic diagnosis

FIG. 3

“polyp”. Every localized swelling of the mucosa in the gastrointestinal tract generally is referred to as “polyp” by the clinicians. In pathologic anatomy a polyp is an autonomous fibroepithelioma then a benign tumor. On the other hand the so-called hyperplastic polyp morphologically is a circumscribed hyperplasia of the mucosa and never a tumor. Of the multitude of problems we want to pick out two special “speech disorders”. The confusion in “borderline lesions” of the mucosa and likewise in “early gastric cancer” seems to be perfect. The former problem from pathologists and gastroenterologists willingly is shifted into the dubious range of “doubtful cases”. A meaningful solution of this controversy is not foreseeable. In the latter problem the “early gastric cancer” we have the well founded suspicion that the use of the japanese nomenclature should improve clinical statistics. At the International Union against Cancer (UICC) an alternative surgico-pathological classification of gastric cancer based on the TNM-system is recommended. This classification is binding upon the gastroenterologist too. T-1-Carcinoma confined to the mucosa, that is an early cancer, and all our efforts have to serve the diagnosis of this earliest malignant lesion. It is our firm conviction that our common worries for the patients let us reach an understanding and live door by door. May be in the near future gastroenterologist and pathologist are “married to each other”.

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